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The Dystonic Brain: Electrophysiological Investigation of Carriers of the DYT1 Gene Mutation.

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PhD Thesis

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ABSTRACT

A mutation in the DYT1 gene on chromosome 9q34 is the commonest cause of young-onset primary dystonia. The penetrance of clinical symptoms is low (only 30-40% of gene carriers manifest dystonia), and occurs in an age-dependent fashion. Mutation carriers who pass their mid-twenties without developing symptoms almost invariably stay symptom free for life. DYT1 mutation carriers therefore provide a unique model with which to study brain function in primary dystonia, and factors that may protect against development of clinical symptoms in those who are genetically susceptible.

This thesis describes the use of electrophysiological techniques to determine 1) if manifesting DYT1 carriers have similar deficits in motor function to non-genetic primary dystonia, and 2) what are the consequences of the DYT1 mutation for motor system physiology in non-manifesting carriers.

We found abnormalities of inhibitory motor circuits at cortical and spinal cord levels in manifesting DYT1 subjects. Surprisingly, we found cortical motor abnormalities of a similar nature and severity in non-manifesting DYT1 carriers, despite their lack of symptoms.

We subsequently demonstrated abnormal reciprocal inhibition in manifesting DYT1 subjects was partially normalised by 1Hz repetitive transcranial magnetic stimulation (rTMS), but that this same stimulus had no effect on

non-manifesting DYT1 subjects or controls. We explored motor system plasticity further in a separate experiment, using a new method of rTMS (theta burst stimulation) as an experimental "plastic force". We found an excessive response to rTMS in manifesting DYT1 subjects and subjects with adult-onset dystonia (torticollis). In contrast we found a sub-normal response to rTMS in non-manifesting DYT1 subjects.

These data suggest that the DYT1 mutation causes abnormalities in cortical motor inhibitory function in all gene carriers, regardless of symptoms, but that a differential sensitivity of the system that underlies synaptic plasticity plays a primary role in determining whether mutation carriers will develop clinical dystonia.

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Statement of Participation in Studies Described

The initial concept for the thesis was generated by Kailash Bhatia and John Rothwell and was then developed by myself. Initial experiments were planned by myself. Dr Ying-Zu Huang played a key role in the design and practical execution of the studies. I participated in all the electophysiological studies described here. Patient ascertainment was conducted by myself as was ethics and R&D application. Analysis and interpretation of data was conducted by myself, Ying-Zu Huang, Kailash Bhatia and John Rothwell. Generation of new hypotheses, particularly those relating to the role of abnormal brain plasticity in the pathophysiology of dystonia was performed by myself, as was planning of the experiments using theta burst stimulation in the assessment of motor system plasticity. Ying-Zu Huang was the major contributer to the development of the new technique of theta burst stimulation. For this reason, the technique is described in an appendix as although I had a secondary role in the development of the technique, it partly reflects my work during the PhD period, and was utilised in one of the experiments described within the main thesis.

Thesis Overview

Chapter 1 will introduce the topic of dystonia from an historical perspective and cover advances in classification of dystonia from an anatomical, aetiological and genetic point of view. DYT1 dystonia will be introduced, and shown to provide a useful model with which to investigate the pathophysiology of dystonia. Hypotheses that were generated with respect to DYT1 mutation carriers are presented.

Chapter 2 provides a literature review regarding the pathophysiology of primary dystonia in general, and also the specific case of DYT1 mutation carriers. This chapter will also explore the concept of brain plasticity, how this can be studied experimentally, and how abnormalities in the regulation of brain plasticity might be relavent to the pathophysiology of dystonia.

Chapter 3 presents the methods used in the experiments described in the thesis.

Chapter 4 presents details of clinical data obtained during patient ascertainment for this study, focussing on unusal phenotypes in DYT1 mutation carriers, and providing a review of the clinical features of previously published cases with the DYT1 mutation.

Chapter 5 describes the first set of experiments where electrophysiological assessments of cortical and spinal motor function were performed in manifesting and non-manifesting DYT1 mutation carriers.

Chapter 6 presents the details of an experiment looking at modulation of spinal reciprocal inhibition using repetitive transcranial magnetic stimulation as an experimental "plastic force" in manifesting and non-manifesting DYT1 mutation carriers and controls.

Chapter 7 presents the details of an experiment to assess cortical motor system plasticity in manifesting and non-manifesting DYT1 gene carriers, subjects with adult-onset dystonia (torticollis) and controls.

Chapter 8 summarises the results of all the experiments and uses them to generate hypotheses that explain the penetrance of clinical symptoms in DYT1 carriers. The data are placed in the context of current knowledge regarding the pathophysiology of primary dystonia in general, and specifically those with dystonia due to the DYT1 mutation. Suggestions are made regarding potential clinical applications of the results from the current research, and the direction which future work could take.

Chapter 1

Introduction to the concept of dystonia and generation of initial hypotheses.

The concept of dystonia has a somewhat chequered history, plagued from the very beginning by implications of a psychogenic rather than organic origin. This is reflected in the one of the first descriptions of dystonia in three siblings with young-onset dystonia. They were described by Schwalbe in 1908 as "familial cramps with hysterical features"(Truong and Fahn, 1988). The term dystonia was coined by Oppenheim in 1911 when he described two patients, one with "dysbasia lordotica progressiva" and the other with "dystonia musculorum deformans". These terms were selected, it appears, depending on the site and functions affected (abnormal gait with twisted postures in the first patient and abnormal muscle spasms and postures of the limbs in the second). Subsequently, the term dystonia came to be used for patients with abnormal postures, particularly for mobile abnormalities rather than fixed postures.

Definition and classification of dystonia

The current commonest definition of dystonia, produced by the Scientific Advisory Board of the Dystonia Medical Research Foundation, is that dystonia is "a syndrome of sustained muscle contraction, frequently causing twisting

and repetitive movements or abnormal postures."(Fahn and Eldridge, 1976). The initial classification system of patients with dystonia was an anatomical one, and, somewhat surprisingly perhaps, this system still has notable clinical relevance. Patients can be classified as focal (one body part only affected), segmental (two contiguous body parts affected), multifocal (two or more non-contiguous body parts affected), hemidystonia (one side of the body affected), or generalised dystonia (two or more contiguous body parts affected plus trunk) (Fahn and Eldridge, 1976). Other terms are used to describe the anatomical distribution of the dystonia, and also, in some cases, are used as a diagnostic label. These terms include: blepharospasm (dystonia affecting orbicularis oculi), oromandibular dystonia, laryngeal dystonia, torticollis (a general term for dystonia affecting the neck, as well as a specific description of head turning caused by dystonia, differentiating it from a head tilt to the side (laterocollis), forward (anterocollis) or back (retrocollis)), writer's cramp (task-specific dystonia affecting the action of writing, but not other tasks.)

When this anatomical system of classification is applied to patients with dystonia, patterns of anatomical involvement that relate to age at onset are revealed. Most important of these is that dystonia with childhood or teenage onset is typically the generalised form, whereas adult-onset dystonia is typically focal (Fahn and Eldridge, 1976). In addition, there appears to be a somatotopic "gradient" of dystonia related to age at onset. Dystonia affecting the feet or legs is only usually seen in those with young-onset primary

dystonia, and even though generalisation of symptoms is typical, involvement of cranio-cervical structures is unusual (Bressman et al., 1998; Bressman et al., 2000). Task-specific dystonias, in particular writer's cramp, tend to have an age at onset of about 30-40 years of age (Jedynak et al., 2001). Cervical dystonia tends to have a later age at onset of about 40-50 years of age (Jahanshahi et al., 1990), with blepharospasm having the latest average age at onset at about 50-60 years of age (Jankovic and Orman, 1984). There is therefore a rostral-caudal gradient of involvement by dystonia that depends on age at onset (Bressman, 2004; Bressman et al., 1994). There are also sex differences in the anatomical distribution of dystonia in that task-specific limb dystonias are commoner in men (Cohen and Hallett, 1988), whereas cranio-cervical dystonias are commoner in women (Jahanshahi et al., 1990; Jankovic and Orman, 1984).

A more recent classification system of dystonia is an aetiological one. In this system, the main separation is between dystonic syndromes that are "primary" and those that are "secondary/heredodegenerative"(Fahn and Eldridge, 1976). In primary dystonia, dystonia is the only clinical feature (+/- tremor), and no structural or neurodegenerative cause is present. Patients with secondary dystonia may have other clinical features apart from dystonia, and a structural or environmental cause is present. In heredodegenerative dystonia, dystonia is typically just part of a wider neurological syndrome which is progressive, and often includes dementia. It should be noted that the diagnosis of primary dystonia does not equate to a diagnosis of "idiopathic"

dystonia: genetic diagnosis of primary dystonia, particularly the young-onset variety, is possible in some cases.

Other groups in this aetiological classification include paroxysmal dystonias, psychogenic dystonia and "dystonia plus" syndromes. These "dystonia plus syndromes" are conditions where dystonia occurs together with other movement disorders, but where there is no secondary or neurodegenerative cause (Bressman, 2004). Only two conditions are included under this heading: dopa-responsive dystonia (DRD) and myoclonic dystonia. In DRD (Segawa syndrome) there is an underlying deficit in the gene encoding guanidine triphosphate cyclohydrolase 1 (GTPCH1), which is a rate limiting step in the metabolism of tetrahydrobiopterin, itself an essential co-factor in the production of dopamine from tyrosine (Ichinose et al., 1994; Ichinose et al., 2001). These patients typically have young-onset limb dystonia with in many cases parkinsonism and mild pyramidal signs. A diurnal fluctuation of symptoms is reported in a proportion of patients with worsening of symptoms throughout the day(Bandmann et al., 1998). Phenotypic variability is common, but in almost all cases a dramatic and sustained response to levodopa is seen.

In myoclonic dystonia, familial early childhood onset dystonia (typically affecting the neck and arms) is accompanied by myoclonus in a similar distribution (Quinn, 1996). The myoclonic jerks are described as "lightning jerks", and alcohol responsiveness is common (Quinn, 1996). Recently,

mutations in the epsilon sarcoglycan gene (SGCE) have been found in a proportion of patients with myoclonic dystonia (Zimprich et al., 2001). The gene shows maternal imprinting, so that offspring receiving a mutant gene from their mother will almost never show symptoms, in contrast to those who receive a mutant gene from their father, where penetrance is almost complete (Grabowski et al., 2003). A summary of ways of classifying dystonia are given in table 1.1.

Table 1.1: Different ways of classifying dystonia

By age at onset	By distribution	By aetiology
Young-onset dystonia (< 28 years) Adult-onset dystonia (> 28 years)	Focal Segmental Multifocal Hemidystonia Generalised	Primary (dystonia only +/- tremor; no neurodegeneration. Dystonia-plus syndromes - Dopa-responsive dystonia - Myoclonus dystonia Secondary - Symptomatic - Heredodegenerative Paroxysmal

Familial forms of dystonia have been recognised for many years, and genetic investigation of such families have revealed a number of possible loci and in some cases particular gene mutations relating to certain types of dystonia.

These are largely summarised in the "DYT" gene classification system, which currently extends from DYT1 to 15 (Nemeth, 2002).

Table 1.2 gives a outline of the conditions covered in the DYT system. Many different aetiological types of dystonia are covered in this classification system (primary, paroxysmal, dystonia-plus, heredodegenerative). Only four genes have actually been identified in these conditions, and currently, commercial testing is only widely available for one of these: DYT1. Some DYT numbers are based on clinical description only (e.g. DYT2 (Gimenez-Roldan et al., 1988; Gimenez-Roldan et al., 1976)), and are not even accompanied by linkage to a particular region. For all these reasons the DYT classification can appear muddled and clinically unhelpful. In its current form, the DYT classification system is simply a list of some (but by no means all) of the genes/loci that have been identified as causing dystonic syndromes, and over and above this, it has little functionality.

Table 1.2: The DYT Classification of Dystonia

DYT Number/name of condition	Age at onset	Clinical Features	Inheritance	Gene	Gene testing available?
DYT1 Oppenheim's dystonia, idiopathic torsion dystonia	Before 25 yrs	Young-onset primary generalised dystonia	AD with low penetrance	GAG deletion in DYT1 gene	Service testing available
DYT2	?	Autosomal recessive young-onset generalised dystonia described in Spanish gypsy family	AR	Unknown	No
DYT3 X-linked dystonia parkinsonism, Lubag	Adult	Progressive dystonia parkinsonism predominantly in Filipino males	X-linked recessive (some females affected)	TAF1	Possibly on a research basis
DYT4 Whispering dysphonia	13-37	Laryngeal dystonia in an Australian family. Torticollis + generalised dystonia seen	AD	Unknown	No
DYT5 Dopa-responsive dystonia, Segawa's disease	Childhood	Young-onset dystonia parkinsonism with diurnal variation and response to levodopa	AD	GTPCH1 gene (Tyrosine hydroxylase deficiency causes a similar, but more severe phenotype)	Yes
DYT6	Variable: average 19yrs	2 Mennonite families with limb and cranio-cervical dystonia	AD	Linkage to 8p21-p22	No
DYT7	28-70yrs	German family with focal craniocervical dystonia	AD	Linkage to 18p	No

DYT Number/name of condition	Age at onset	Clinical Features	Inheritance	Gene	Gene testing available?
DYT8 Paroxysmal non-kinesogenic choreoathetosis	Childhood-teenage years.	Attacks of dystonia and chorea precipitated by alcohol, coffee, fatigue.	AD	Myofibrillogenesis regulator 2 gene	Possibly on a research basis
DYT9	Childhood, teenage years	Episodic chorea and ataxia with progressive interictal spasticity	AD	Linkage to 1p	No
DYT10 Paroxysmal kinesogenic choreoathetosis	Childhood-teenage years	Brief attacks of chorea and dystonia precipitated by sudden movement	AD	Linkage to pericentromeric region of chromosome 16 in some families	No
DYT11 Myoclonus dystonia	Childhood	Myoclonus +/- dystonia responsive to alcohol	AD with maternal imprinting	Epsilon sarcoglycan gene	Yes
DYT12 Rapid-onset dystonia parkinsonism	Variable	Dystonia and parkinsonism developing over hours/days, often following infection	AD	ATP1A3 gene mutations	Possibly on a research basis
DYT13	Childhood-adult	Cranio-cervical dystonia in one Italian family	AD	Linkage to 1p36.13-36.32	No
DYT14 Dopa-responsive dystonia	Single case: onset age 3	Dopa-responsive dystonia parkinsonism	?	Linkage to chromosome 14q13, but outside region of GTPCH1 gene	No
DYT15	Childhood-adult	Myoclonus +/- dystonia responsive to alcohol	AD	Linkage to 18p11	No

DYT1 dystonia – from primary torsion dystonia to the DYT1 gene

Amongst familial forms of primary dystonia, the most common pattern is of young-onset (late childhood/early teens) dystonia, starting in a limb and then becoming generalised, but usually sparing the cranio-cervical region. This pattern has been recognised for many years and became known as primary or idiopathic torsion dystonia (Fahn and Eldridge, 1976). Since the delineation of this clinical phenotype it was recognised to have a high prevalence amongst the Ashkenazi Jewish population (Bressman et al., 1994) , and it was a source of dispute whether this was even the same condition as that which occurred amongst non-Jewish people (Burke et al., 1986). The mode of inheritance was also a source of debate – a recessive inheritance was most often favoured, although dominant inheritance with reduced penetrance was also suggested (Bressman et al., 1988).

Linkage studies in a large number of Ashkenazi Jewish families identified a candidate region on chromosome 9q34, and also confirmed the inheritance to be autosomal dominant with low penetrance of approximately 30%(Bressman et al., 1994). The increased prevalence in the Ashkenazi Jewish population is thought to be due to a "founder effect", i.e. a population bottleneck that occurred in the past. The origins of the founder effect in respect to the DYT1 mutation have been traced back to the 1600s, where pogroms against the Jewish community in Eastern Europe created a small population in which interbreeding occurred (Risch et al., 1995). Later, non-Jewish families with

the idiopathic torsion dystonia phenotype were linked to the same region of chromosome 9, and in 1997 the responsible mutation was identified in the DYT1 gene (also known initially as the TOR1A gene) (Ozelius et al., 1997). This mutation is a single GAG deletion that removes a glutamate residue from close to the ATP binding end of the protein torsin A (Ozelius et al., 1997).

The DYT1 phenotype

The phenotype associated with this mutation was initially thought to be simply that of typical idiopathic torsion dystonia with childhood limb-onset dystonia that generalises in most cases and then stops progressing, with cranio-cervical involvement not seen. As DYT1 testing became more widespread, a significant phenotypic variability became apparent. The variability of this phenotype is analysed in detail in the clinical study described in chapter 3.

The issue of penetrance of clinical symptoms in DYT1 gene carriers.

A feature of the DYT1 mutation that is critical to the design of the studies presented here is that of its low age-dependent penetrance. Penetrance of clinical symptoms in DYT1 mutation carriers is approximately 30%, and almost all those who are going to manifest symptoms will do so before the age of 25. DYT1 mutation carriers therefore present a unique opportunity to the researcher with an interest in the pathophysiology of dystonia. Firstly, in

contrast to many other genetic forms of dystonia, the mutation is relatively common, and therefore it is feasible to collect a cohort of a reasonable size in whom to conduct experiments. Secondly, in contrast to other patients with primary dystonia, patients with dystonia due to the DYT1 mutation have a common underlying cause despite variable severity of symptoms, and this helps to eliminate possible bias in experiments from a lack of homogeneity of subjects in terms of underlying aetiology. Thirdly, penetrance is low, providing a cohort of individuals who carry the DYT1 mutation, but who do not manifest symptoms. Due to the known age-dependency of manifestation of symptoms, the researcher can be reasonably confident that non-manifesting gene carriers over the age of thirty will not manifest symptoms in the future and thus can be considered as truly different from manifesting gene carriers (Bressman et al., 2000).

Aims and Hypotheses

We had two main aims. Our first aim was to use electrophysiological techniques to probe the function of the motor system in manifesting DYT1 carriers. For the reasons outlined above, this group of patients are of interest with regard to understanding the pathophysiology of primary dystonia, and have not previously been studied electrophysiologically. Our second aim was to use the unique natural model provided by the low, age-dependent penetrance of DYT1 dystonia to try to understand the mechanisms that drive the development of clinical symptoms in genetically susceptible individuals

We set out to test four main hypotheses in these initial experiments:

1. That manifesting gene carriers would have similar abnormalities in cortical and spinal motor inhibitory function as previously described in non-genetically characterised primary dystonia.

2. That non-manifesting gene carriers are asymptomatic as the DYT1 mutation has no consequences for them (perhaps as it is inactivated by some mechanism).

3. That electrophysiological abnormalities are present in non-manifesting gene carriers that affect similar systems to those seen in manifesting gene carriers, but are of a lesser severity, and do not reach the threshold for clinical symptoms to be produced.

4. That non-manifesting gene carriers have only a sub-set of the pathophysiological abnormalities present in manifesting gene carriers, and these are not sufficient to produce clinical symptoms.

Based upon data from the experiments described in chapter 4, further hypotheses were generated leading to experiments described in chapters 5 and 6:

1. That carriers of the DYT1 mutation who develop dystonia will have an excessive response to an experimental plastic force.
2. That subjects with adult-onset non genetically characterised dystonia (torticollis) will have an excessive response to an experimental plastic force.
3. That carriers of the DYT1 mutation who do not develop dystonia will have a sub-normal response to an experimental plastic force.

Chapter 2

The Pathophysiology of Primary Dystonia

The development of the clinical concept of dystonia outlined in the last chapter was paralleled by a developing understanding of the underlying pathophysiology of dystonia in general and primary dystonia in particular. As the clinical concept of dystonia has moved from anatomy to aetiology to genetics, so has the pathophysiological understanding. The last step has been particularly fuelled by the discovery of the DYT1 gene mutation. This has opened an entirely new field of study in dystonia research: the function of torsin A and its role at a molecular level within the cell. It is likely that this work will lead to better understanding of primary dystonia in general.

The basal ganglia and dystonia

Simply at a conceptual level, dystonia, as a movement disorder, was thought to arise from dysfunction within the basal ganglia. Indeed, the observation that dystonia could occur secondary to lesions of the basal ganglia (Bhatia and Marsden, 1994) strengthened the view that in primary dystonic conditions (where no basal ganglia damage was seen) there was likely to be a functional disturbance of basal ganglia modulation of cortical motor pathways.

Cortical function in dystonia

A variety of techniques have been employed to study the function of the motor system at a cortical level in primary dystonia.

Transcranial magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive method of stimulating cortical neurons. A magnetic field generator drives a current of approximately 200μs with a peak amplitude of 8,000 A through an induction coil placed on the scalp. The current creates a time-varying magnetic field perpendicular to the coil. The magnetic field penetrates the skull and then induces an eddy current parallel to the coil in the brain. This current is capable of stimulating the brain and can produce descending volleys in the corticospinal pathway which can be recorded using surface EMG from the appropriate muscles.

A figure of eight coil is often used to provide a more focal stimulus than that obtained from a simple circular coil. If a figure of eight coil is held such that the TMS pulse causes current to flow in an posterior-anterior direction perpendicular to the central sulcus, then this tends to provide the lowest threshold for stimulation and appears to activate corticospinal neurons trans-synaptically (Di Lazzaro et al., 2001). As stimulation intensity is increased, a rising proportion of activation occurs directly.

The tendency for trans-synaptic activation means that the response to TMS is altered by the excitability of these synapses at the time of stimulation. Therefore TMS is useful as a technique to explore the integrity and excitability of motor pathways, and can be applied before and after an intervention to determine whether a change in synaptic excitability has occurred.

In order to understand the literature on TMS techniques applied to patients with dystonia outlined below, certain aspects of TMS methodology deserve particular comment. First is the concept of the motor "hot spot". This is the area on the scalp over with TMS of a particular intensity produces the largest motor evoked response (MEP) from the target muscle. Due to ease of stimulation, the most commonly used target muscle is the first dorsal interosseus (FDI). Surface EMG is recorded from FDI during stimulation, and once the "hot spot" has been identified it is marked on the scalp. Second is the concept of resting and active motor thresholds. Resting motor threshold (RMT) is the intensity of stimulation that produces no detectable EMG response from the target muscle when that muscle is relaxed. Active motor threshold (AMT) can be defined as the intensity of stimulation that produces an EMG response of less than 200μV in less than five out of ten trials when the target muscle is voluntarily contracted. Typically feedback is given to the subject to maintain this voluntary contraction at a set level (about 20-30% of maximal contraction).

A variety of TMS methodologies have been applied to patients with dystonia and have provided important insights into dystonia pathophysiology.

Input/Output curves

Although no differences have been found in thresholds for activation of muscles in dystonia subjects compared to controls, differences have been observed in the input/output relationship in response to TMS. In these experiments, the RMT for a particular individual is established, and then TMS pulses at increasing intensity of stimulation based on percentages of RMT are delivered, and the size of the resulting MEP recorded. This provides an input/output curve where MEP size is plotted against magnitude of TMS intensity. In dystonic subjects, a significantly enhanced input/output curve is found, such that MEP size is significantly larger for a given input compared to control subjects (Ikoma et al., 1996; Mavroudakis et al., 1995). This finding has been interpreted as demonstrating increased motor system excitability in dystonia. A difficulty with this interpretation (and one that interferes with the interpretation of many experiments in those with dystonia) is that muscle activity directly influences the size of MEP produced from TMS of a given intensity of stimulation. Muscle activation (or even thinking about muscle activation) increases MEP size. This means that scrupulous monitoring of baseline EMG in the target muscle (and perhaps ideally the adjacent muscles too) is required in order to prevent this possible artefact in experiments in

dystonic subjects, who may have a significant amount of involuntary muscle activity.

Short Intracortical inhibition and facilitation.

Kujirai et al (Kujirai et al., 1993) developed a paired pulse TMS technique which is thought to stimulate different populations of inhibitory and excitatory interneurons, and provides measures of their excitability: intracortical inhibition and facilitation. The standard method explores the influence of a sub-threshold "conditioning" pulse on the size of the MEP produced by a subsequent "test" pulse. The intensity of the test pulse is usually set to achieve an MEP of about 1mV when given alone. The conditioning pulse is then given at different time intervals prior to the test pulse.

In studies with normal subjects, the conditioning pulse given 1-5ms prior to the test pulse causes a reduction in the resulting MEP (Kujirai et al., 1993). This effect is known as short interval intracortical inhibition (SICI). The effect is enhanced by GABAa agonists, NMDA receptor blockers and dopamine agonists and is blocked by dopamine antagonists (Ziemann et al., 1996a; Ziemann et al., 1996b; Ziemann et al., 1996c). It is proposed that SICI is a GABAa mediated pathway that has an inhibitory influence of corticospinal tract excitability.

There is a cross-over or intermediate period of response when the conditioning pulse is given between 6 and 9ms prior to the test pulse, where little effect is seen on the resulting MEP. At interstimulus intervals (ISI) of 10-20ms an increase in the size of MEP is typically seen, a phenomenon known as intracortical facilitation (Kujirai et al., 1993). The mechanism of this effect is unclear at the present time. It can be modified by rTMS independently of SICI indicating that different pathways underlie the two phenomena (Peinemann et al., 2000). Currently it is thought most likely to be a glutamate mediated event (Ziemann et al., 1996c).

SICI can be influenced by the intensity of the conditioning pulse. SICI is recordable using conditioning pulse intensity of 60% RMT at and ISI of 3ms. The magnitude of the effect increases as the intensity of the conditioning pulse is increased, and reaches a maximum at approximately 90% of RMT or 80% AMT(Kujirai et al., 1993; Ziemann et al., 1996d). Further increases in intensity lead to progressively less SICI. Although less certain, it may be that the optimum intensity for producing ICF is slightly higher than that for SICI.

There have been a number of studies exploring SICI and ICF in patients with primary dystonia (Berardelli et al., 1998b). The most consistent finding has been of a reduction in SICI in dystonic individuals (Ridding et al., 1995). This has been interpreted as a failure of inhibitory control of motor pathways, which could lead to problems in focusing desired movement and could lead to unwanted muscle activity (Berardelli et al., 1998b). As with input-output

experiments, measurement of SICI (and ICF) is hampered by muscle contraction – it will tend to reduce SICI and increase ICF. However, reductions in SICI have been demonstrated using target muscles that are not involved by dystonia (e.g. FDI in patients with cervical dystonia). However, the question remains as to the effect on SICI and ICF of even distant involuntary muscle activity. Abnormalities in ICF have been more variable, but some studies have reported increases in ICF (Sommer et al., 2002a), again in keeping with an over-excitable corticospinal system.

Silent period

The silent period is a period of EMG silence that occurs following a TMS shock delivered over representative area of cortex of a voluntarily contracting muscle. Typically, constant sub-maximal contraction of FDI is achieved via the use of auditory or visual feedback. A TMS pulse is then delivered over the motor hotspot relating to the FDI at an intensity of 110-130% of RMT (with a higher stimulus intensity possibly providing a more consistent result (Orth and Rothwell, 2004)). A temporary break in EMG activity will occur which is called the silent period. This can be measured in a variety of ways, but perhaps most reliably by measuring the interval between the onset of the stimulus artefact and the first recovery of EMG activity.

Studies in normal subjects typically find the silent period to be 100-120ms in length. Via examining the effect of GABAa and GABAb antagonists and

agonists, Ziemann et al have proposed that the SP is a GABAb mediated process (Ziemann et al., 1996c). There is likely to be a small additional spinal component (Inghilleri et al., 1993).

In dystonic subjects a number of studies have found a shortened silent period (Berardelli et al., 1998b). This would suggest a deficit in GABAb mediated inhibition in dystonia.

Pre-movement potentials

Two types of pre-movement potential have been recorded in dystonia: the Bereitschaftspotential (BP) and the contingent negative variation (CNV). The BP is a slow rising (negative) EEG potential that begins 1.5-2 seconds prior to a self-paced voluntary movement. Initially the potential is diffuse and bilateral (NS1: bilateral primary and supplementary motor area activity), and then becomes lateralised (NS2: contralateral primary motor area activity). The CNV is recorded in a different fashion. Here EEG is recorded between a warning cue and a "go" signal to perform a particular movement. During the gap between the two stimuli, a slow negative potential is recorded which, like the BP, is at first bilateral and then lateralises to the representative hemisphere for the planned movement.

Studies of the BP and CNV in patients with primary dystonia have found a reduction in amplitude of the potentials compared to normal subjects. In most

experiments this abnormality was only present when the planned movement involved a body part affected by dystonia (Deuschl et al., 1995; Ikeda et al., 1996; Van der Kamp et al., 1995).

Brainstem motor function in dystonia.

Blink reflexes

At a brainstem level, the most studied circuit is the blink reflex and in particular the blink reflex recovery cycle.

If a stimulus is delivered to the supraorbital nerve of sufficient intensity, an ipsilateral contraction of orbicularis oculi will occur (R1 component) followed by a bilateral contraction of orbicularis oculi (R2 component).

The blink reflex recovery cycle is typically assessed by delivering paired stimuli to the supraorbital nerve a different interstimulus intervals, and comparing the size of the R2 response to that obtained when a single stimulus is given. In normal subjects interstimulus intervals of less than 750ms typically result in a significantly reduced R2 size. In certain types of dystonia (blepharospasm, cervical dystonia, generalised dystonia) this recovery cycle is enhanced such that the R2 component is large even at ISIs of 250-500ms (Berardelli et al., 1985; Eekhof et al., 1996; Nakashima et al., 1990; Tolosa et al., 1988). Although abnormalities of the blink reflex recovery

cycle are clearly seen in some types of dystonia that clinically do not involve orbicularis oculi, some studies have failed to find blink reflex recovery cycle abnormalities in other types of dystonia such as segmental dystonia not involving the head or neck(Nakashima et al., 1990).

Other brainstem abnormalities.

The masseteric inhibitiory reflex is obtained by stimulating the masseteric nerve during voluntary muscle contraction. This masseteric silent period has two phases (SP1: early; SP2: late) and in normal subjects has a recovery period such that the second of two paired stimuli of short ISI will fail to cause an SP2 response. As with the blink reflex, studies in dystonia have found that this reflex recovery cycle is more excitable than normal subjects. This is true of patients with oromandibular dystonia as well as those without any clinical dystonic involvement of jaw muscles(Pauletti et al., 1993).

Vestibular abnormalities have been reported in those with torticollis, although it is not clear if these are primary to the dystonia or are secondary to a prolonged period of abnormal head position. The latter seems most likely as in general studies have found a suppression of normal vestibular responses (Bronstein and Rudge, 1988).

Spinal motor abnormalities in dystonia

Although primary dystonia by definition does not present clinically with signs of corticospinal or radicular dysfunction, electrophysiological testing has revealed deficits in spinal reflex control.

The H reflex (described by Hoffman in 1918) is effectively an electrical method of mimicking the tendon tap reflex, although one which bypasses the muscle spindle as the afferent nerve fibres are stimulated directly. Although the H reflex has been obtained from a variety of muscles, flexor carpi radialis and soleus are in general the most reliable.

The H reflex in those with dystonia has been found to have a shorter recovery cycle compared to normal subjects when stimuli are given with ISI of 200ms. This finding is not just the case for those with dystonia involving the limb assessed, but also in those with craniocervical dystonia, without clinical involvement of the limbs.

Reciprocal inhibition is a technique that explores experimentally the issue of interaction between agonist and antagonist muscles: an issue of central importance to the pathophysiology of dystonia. Reciprocal inhibition (RI) assesses the interaction between stimulation of the radial nerve supplying the extensor muscles of the forearm and the H reflex produced by stimulation of the median nerve. At particular interstimulus intervals (ISIs), a reduction in

the size of the H reflex occurs in normal subjects (Day et al., 1984). The first phase of inhibition, occuring at ISIs of approximately 0msec, is mediated by a glycinergic disynaptic inhibitory pathway (Day et al., 1984). The second phase of inhibition, occuring at ISIs of 10-20msec, is thought to be due to presynapic Ia inhibition of afferent fibres that mediate the H reflex (Berardelli et al., 1999). The origin of the third phase of inhibition, occurring at ISIs of 70-500msec, is less well known and might go through the polysynaptic long latency stretch reflex pathway (Chang et al., 1997).

Studies in those with dystonia (both with dystonic involvement of the tested limb or without) have found a reduction of inhibition that occurs at the 2nd and 3rd phases.

Kinematic studies in dystonia have demonstrated overlapping activity of agonist and antagonist muscles and a slowness (but not fatiguing) or movement. The normal triphasic pattern of agonist and antagonist activity appears to be lost, and movements are characterised by a high degree of variability (van der Kamp et al., 1989).

Sensory system abnormalities in dystonia

The presence of the "sensory geste" in those with dystonia has long been viewed as a pointer to a possible role for abnormal sensory function in dystonia.

Early studies found inconsistent abnormalities in the late (N30) component of the somatosensory evoked potential (Onofrj et al., 1995; Reilly et al., 1992). Certainly, modification of sensory input can affect dystonia symptoms. Afferent blockade by local anaesthetic can reduce the severity of dystonic symptoms (Yoshida et al., 2002). There is an abnormal response to vibration of the affected and unaffected body parts of those with dystonia (Yoneda et al., 2000).

In an elegant study, Tinnazi and colleagues examined the amplitude of the N20 component of the SEP obtained in normal and dystonic subjects when the median or ulnar nerve were stimulated separately, or when both were stimulated together (Tinazzi et al., 2000). They hypothesised that in normal subjects the N20 from the paired SEP would be smaller than the arithmetical sum of the N20 derived from unpaired stimulus of the median and ulnar nerve due to sensory "gating", a phenomenon thought to help the integration of sensory input in the brain.

This "gating" of sensory input was indeed found in normal subjects, but in those with dystonia, the N20 from paired stimuli was much greater than normal subjects, and close to the arithmetical sum of the single stimuli. This would suggest that the sensory system in dystonia fails to integrate complex sensory information.

Transcranial magnetic stimulation has been used to study sensorimotor integration in dystonia (Abbruzzese, 2001). In this study 12 patients with focal hand dystonia were compared with 16 normal subjects. All subjects received electrical stimulation of the median nerve at intervals of 50, 200, 600 or 1000ms prior to a TMS shock delivered over the hand motor area correcponding to abductor policis brevis. In normal subjects an inhibitory effect of median nerve stimulation was seen on MEP, maximal at an ISI of 200ms. In contrast, subjects with focal hand dystonia showed no such inhibition of MEP size, and indeed demonstrated facilitation instead. This study demonstrates abnormal interaction between sensory input and motor output in dystonia.

Imaging in dystonia

Structural imaging

Simple structural imaging is normal in primary dystonia. High field studies in dystonia (spasmodic torticollis) have identified prolonged T2 relaxation times in the lentiform nucleus compared to normal subjects (Schneider et al., 1994).

More recently, voxel-based morphometry has been used to look at brain anatomy in dystonia. In one study of 10 patients with torticollis (Draganski et al., 2003), voxel-based morphometry revealed an increase in gray matter density bilaterally in the motor cortex and in the cerebellar flocculus and

unilaterally in the right globus pallidus internus. In addition, a decrease in gray matter density was observed in the right caudal supplementary motor area as well as in the right dorsal lateral prefrontal and visual cortex.

A more recent study of 36 patients with focal hand dystonia, again using voxel-based morphometry, revealed significant bilateral increase in gray matter in the hand representation area of primary somatosensory and, to a lesser extent, primary motor cortices. The finding of bilateral abnormalities in those with unilateral dystonia led the authors to suggest that these abnormalities might to some extent be primary (Garraux et al., 2004).

Functional imaging

Studies of regional blood flow in dystonia have, in general, found no differences compared to normal subjects. There have been suggestions of differential glucose metabolism in those with dystonia, and in particular for putaminal hypermetabolism, but results are inconsistent (Berardelli et al., 1998b).

Regional blood flow changes have been examined during movement. Ceballos-Baumann and colleagues examined regional blood flow in normal and dystonic patients during paced freely selected movements of a joystick and during writing. There was underactivity in primary and supplementary

motor areas, and excessive activation of prefrontal, cerebellar, insula and parietal cortex (Ceballos-Baumann and Brooks, 1998).

A study in seven patients with focal arm dystonia using magnetic resonance spectroscopy found a reduction of GABA in the sensorimotor cortex and lentiform nucleus contralateral to the affected hand, but no such change on the ipsilateral side (Levy and Hallett, 2002).

DYT1 dystonia: pathophysiology

Molecular studies

Torsin A is a protein whose function is still not known. It is widely expressed throughout the body, and indeed has its highest levels in the liver. However, DYT1 dystonia is the only known disease to arise from defects in the DYT1 gene, and those with DYT1 dystonia have not been found to have other organs or systems affected directly by the genetic defect.

Within the brain, torsin A has a specific localisation. Normal human brains have been studied post mortem looking for DYT1 mRNA. The neocortex was found to have a largely homogenous low level of DYT1 RNA. There was a high level found in the hippocampus, particularly the dentate gyrus and also in the substantia nigra pars compacta (specifically in the melanised i.e.

dopaminergic neurons). Little DYT1 mRNA was found within the rest of the basal ganglia (Augood et al., 1999; Konakova et al., 2001).

Torsin A is an ATP-binding protein, and part of the AAA+ family of proteins (*A*TPases *A*ssociated with a variety of cellular *A*ctivities). This superfamily of proteins is highly conserved across species and share an Mg^{2+}-ATP binding domain and form a six-membered ring structure (Breakefield et al., 2001). A typical role for these AAA+ proteins is in chaperone function, mediating conformational change in other proteins. Other members of the family include heat shock proteins (Breakefield et al., 2001).

One role of such proteins that may have relevance for the pathophysiology of dystonia is in controlling spatially and temporally membrane fusion processes. This may have relevance to later discussion on plasticity in dystonia and DYT1 where the temporal and spatial control of membrane fusion events between neurotransmitter vesicles and the post-synaptic membrane is likely to have consequences for the ease in which plastic change can be produced at synapses. In this regard, a recent study of cellular localisation of overexpressed mutant torsin A is of considerable interest (Misbahuddin et al., 2005). This study confirmed the localisation of torsin A to the endoplasmic reticulum, but in addition identified that the includsions formed by the mutant torsin A were immunoreactive for vesicular monoamine transporter 2 (VMAT2). VMAT2 expression is important for the exocytosis of bioactive monoamines in neurons. Abnormal processing, transport, or entrapment of

VMAT2 within the mutant torsinA membranous inclusions, therefore, may affect cellular dopamine release.

Torsin A is an endoplasmic reticulum luminal protein(Hewett et al., 2003), and as with other members of the AAA+ family is hypothesised to form a six membered ring structure within the lumen of the endoplasmic reticulum. Theoretical modelling of the effect of the common DYT1 mutation on this structure finds that the mutation could either disrupt closure of the ring, or interaction with the partner protein. With equal levels of mutant and wild-type protein within the cells, activity is hypothesised to fall to less than 2% of normal (Breakefield et al., 2001).

Torsin A may have a role in neuroprotection events within cells, and mutant torsin A may both interfere with this function or even be damaging to the cell itself (Kuner et al., 2003; Shashidharan et al., 2004). Torsin A is a component of Lewy bodies, perhaps further indicating that it has a neuroprotective function (Shashidharan et al., 2000). Overexpression of mutant torsin A causes the formation of "whorls" within the ER (Hewett et al., 2000), although these are not seen at levels of mutant torsin A found in DYT1 carriers. Torsin A has also been proposed as a factor in stabilising various protein kinases which in turn phosphorylate microtubule associated proteins such as tau. In this way, torsin A may help to maintain site-directed polarization and control neurite outgrowth in cells(Ferrari-Toninelli et al., 2004).

In conclusion, these molecular studies demonstrate a particular location for torsin A within the brain, and suggest a role for the protein in protein chaperoning, membrane interactions, monoamine vesicular function and control of neurite outgrowth.

Pathological studies in DYT1

To date, there are few pathological studies in DYT1. Initial studies found no pathological abnormalities (Walker et al., 2002). One study has found evidence of increased dopamine turnover in the brains of those with the DYT1 mutation, as indicated by a significant increase in the striatal 3,4-dihydroxyphenylacetic acid/dopamine ratio (Augood et al., 2002).

A recent study of the brains of 4 DYT1 positive patients found perinuclear inclusion bodies in the midbrain reticular formation and the periaqueductal grey matter. The inclusions were located in the pedunculopontine and cuneiform nuclei. They stained positively for ubiquitin and torsin A. No inclusions were found elsewhere in the brain (McNaught et al., 2004). The significance of these findings is uncertain at the present time. It seems unusual that inclusions should be localised solely to the brainstem. Clearly brainstem motor function as revealed by electrophysiological study is abnormal in dystonia. However, it seems difficult to explain the full clinical spectrum of dystonia on the basis of brainstem pathology alone.

Structural imaging studies

Patients with DYT1 dystonia were not found, on routine clinical imaging, to have any consistent abnormality in brain structure. However, in parallel with imaging in other forms of primary dystonia, more advances structural imaging techniques have now been applied to DYT1 mutation carriers. In a recent study (Carbon et al., 2004), 12 DYT1 mutation carriers (8 manifesting and 4 non-manifesting) were subjected to diffusion tensor imaging to assess microstructural white matter changes. Fractional anisotropy (FA) values, which are thought to reflect microstructural features such as fibre integrity and coherence, were calculated for subjects and the results compared with controls. Reductions in FA were found in DYT1 subjects in the sensorimotor cortex, the posterior splenium and in the right pre-central gyrus. Comparison of the manifesting and non-manifesting DYT1 groups revealed a greater reduction in the sensorimotor cortex FA in manifesting compared to non-manifesting subjects.

Functional imaging studies

In 1998, Eidelberg and colleagues used flurodeoxyglucose PET to compare patterns of regional glucose metabolism in manifesting and non-manifesting patients. At rest they found similar, abnormal patterns of increased metabolism in the lentiform nucleus, cerebellum and supplementary motor

areas which were hypothesised to represent abnormal patterns of activation in cortico-striato-cortical loops. It was interesting to note that the patterns of abnormality were similar in manifesting and non-manifesting individuals despite their clinical differences (Eidelberg et al., 1998). FDG PET is not easily amenable to quantification, and therefore the question of degree or severity of abnormality is not answered by this study.

The same group have recently published a further functional imaging study in DYT1, this time using [11C] raclopride PET, a ligand that binds to the D2 receptor (Asanuma et al., 2005). Only non-manifesting DYT1 carriers were studied, and a reduction in D2 binding of about 15% was found in the caudate and putamen. This reduction was less than that observed previously in non-DYT1 primary dystonia, and the authors propose a possible threshold effect of D2 receptor loss on the development of clinical dystonia.

In conclusion, a wealth of electrophysiological and imaging data exists in patients with primary dystonia. The overall impression is of a reduction in motor inhibitory circuit activity/function evident at many levels of the nervous system, but most likely with its origins in the basal ganglia. Sensory system function is certainly not normal in dystonia, but it is still unclear whether this is a primary feature of dystonia or its consequence.

The discovery of the DYT1 mutation has enabled study of a select group of patients with primary dystonia with a homogenous aeitiology. Molecular

studies have provided intriguing insights into the possible role of torsin A within the cell and the problems that mutant torsin A might provide for normal cell function. Certainly the localisation of torsin A to dopaminergic cells in the substantia nigra provides further evidence for the importance of the basal ganglia in primary dystonia. As will be expanded later, there are reasons to explore further the ways in which torsin A might affect vesicular function at synapses and the effect mutant torsin A might have on temporal and spatial (and quantal) neurotransmitter release.

Functional imaging studies in DYT1 have provided clues that clinically normal individuals who carry the DYT1 mutation have abnormalities in brain structure and function. What is not clear from these studies is how these abnormalities, which are also present in manifesting DYT1 gene carriers, relate to the appearance of clinical symptoms.

Brain plasticity and dystonia

The data presented above provides a simple model for primary dystonia based on the concept of a poorly inhibited motor (or sensori-motor) system. However, abnormalities of intracortical inhibition, silent period, reciprocal inhibition and abnormal patterns of brain metabolism seen on PET are common findings in a number of movement disorders, and do not seem to be capable of encompassing many of the unusual clinical features of dystonia. A more complete explanation of the pathophysiology of dystonia might be

gained by considering a primary role for abnormal plastic changes in the brain as the cause of the syndrome. First, let us consider some of the basic science behind the concept of brain plasticity, and how it is possible to examine it experimentally.

Plasticity, in regard to neural systems, can be defined as the ability of a system to change in response to stimuli (internal or external), and then to maintain that changed state until further stimuli occur. The ability of the nervous system to undergo such plastic changes can be demonstrated experimentally by the observation of change in the functional organisation of cortical areas in response to stimuli. With regard to the motor system, these plastic changes can be demonstrated by observing changes in motor "maps" in the primary motor cortex (M1) in response to pathological or physiological interventions. Thus, section of facial nerve supply to whiskers in rats can produce rapid spread or "bleeding" of the adjacent forelimb representation into the vibrissae area, so that forelimb movement occurs via stimulation of the whisker cortical area (Donoghue et al., 1990). Learning of motor skills in primates and humans is associated with expansion of cortical representation of the body parts involved in the motor task. Such changes may only occur for the practice of skilled movements, and not simply repetitive simple movements (Kleim et al., 1998), a point with potential relevance for the aetiology of task-specific dystonia.

While there is consensus that plasticity in the motor system can and does occur, the mechanism of plastic changes remains the subject of intense debate. From an anatomical point of view, the motor cortex provides a structure that would seem to allow plastic changes to occur, with spreading horizontal fibres (in particular in layer II/III). These would appear to facilitate the development of networks across the motor cortex (and non-motor areas as well) in response to stimulation (Gilbert et al., 1996).

Donald Hebb is credited with the development of the most widely accepted theory regarding plastic changes in the nervous system: "Hebbian plasticity". Simply stated, the theory is that increases in synaptic strength occur when there is concurrent activity in pre and post synaptic cells (Hebb, 1949). This theory would seem only to allow for increases (and hence eventual saturation) of synaptic strength. In response to this, Stent (Stent, 1973) proposed an addition to this rule, namely that uncorrelated activity in pre- and post-synaptic cells would tend to lead to a decrease in synaptic strength.

This "covariance" theory of synaptic plasticity allows bidirectional changes in excitability at synapses, but would lead to the rather unphysiological consequence that excitability at synapses would tend either to increase exponentially, or to sink to zero – these simple rules do not appear to allow a gradation of synaptic strength. Homeostatic mechanisms are proposed that maintain the stability of the network, perhaps by "resetting" the boundaries of excitability within a particular network. This can be demonstrated

experimentally in motor learning experiments in rats where extended training on a particular task shifted the synaptic modification range of the target cells upwards, so that instead of becoming saturated, further increases in synaptic strength could occur (Rioult-Pedotti et al., 2000). Effectively, it appears that more the previous history of activity at the synapse, the more difficult it is to potentiate it.

Proposed mechanisms of synaptic plasticity

There are a variety of proposed components to synaptic plasticity. Long-term potentiation and depression (LTP/LTD) are the most well known of these, but it is likely that a number of overlapping processes occur at the synapse that allow changes in synaptic function to occur in response to stimulation.

Presynaptic plasticity

Short lasting changes in synaptic efficiency (lasting milliseconds to seconds) can occur due to changes at the pre-synaptic level. Short term potentiation and depression are thought to relate to changes in the amount and/or probablility of transmitter release. Reduction in calcium influx to the pre-synaptic bulb appears to induce STD, whereas increases in intra-cellular calcium concentrations appears to favour STP induction (De Camilli et al., 2003). The effects of calcium may be direct, or mediated via calcium dependent kinases, which in turn alter the activity of synapsin, a protein that

alters vesicle mobility(Picconi et al., 2003). When activated, synapsin favours release of vesicles and experimentally causes STP induction. STD might occur via the reduction of phosphoylated synapsin(Geppert et al., 1997). Other mechanisms of pre-synaptic plasticity include availability of vesicles (they are stored in an immediate and long-term pool), growth of the bouton, and number of boutons.

Post-synaptic mechanisms

There are mechanisms that allow short-term post-synaptic plasticity, either to favour increases or decreases in synaptic function. AMPA receptors, which are blocked by intracellular polyamines, have this block removed when depolarisation occurs, allowing short term facilitation of the post-synaptic response to excitation (Rozov and Burnashev, 1999; Rozov et al., 1998). Desensitization is an opposing process where a proportion of ligand-gated channels are rendered inactive for a short period after exposure to an agonist (Jones and Westbrook, 1996).

Brain slice preparations have been extensively used to study the mechanisms and controls over synaptic plasticity. In the majority of such experiments in the motor cortex, stimulating micro-electrodes are placed into layer II/III and field potentials are recorded for a given level of stimulus before and after a conditioning stimulus. The measured field potentials (FP) are analogous to the excitatory post-synaptic potential (EPSP). Changes in the FP in response to

conditioning provide a direct measure of changes in synaptic strength. This approach allows manipulation of conditioning stimuli and physiological conditions in order to better understand the mechanisms of synaptic plasticity.

Long-term potentiation (LTP) of synaptic strength can be reliably produced in animal brain slice preparations by high frequency direct electrical stimulation (Bliss and Lomo, 1973). This has led to the extensive study of the molecular changes that occur following LTP induction and of methods to alter the direction or magnitude of the change in synaptic strength. In summary, these studies have found LTP to, in general, be an NMDA receptor dependent process, so that pharmacological(Morris, 1989) or genetic(Sakimura et al., 1995) blockade of these receptors leads to failure of LTP induction. GABA clearly plays an important role in LTP induction, in particular in the motor cortex, where reduction of GABA using the GABA antagonist bicuculline, is typically required for successful induction of LTP(Chen et al., 1994). Other important components of successful LTP induction include rate of change in intracellular calcium levels (Yang et al., 1999) and the presence of dopamine, without which LTP induction is impaired (Kusuki et al., 1997)). The majority of these studies have been in hippocampal tissue, although the role of GABA in LTP has been most often demonstrated in motor cortical tissue.

Long-term depression is a more recently discovered phenomenon, and is typically produced by prolonged periods of low-frequency stimulation (LFS)

(Lynch et al., 1977). LFS can abolish LTP (a process known as depotentiation), and has been proposed as a mechanism of "forgetting" (Picconi et al., 2003).

Experimental protocols capable of inducing LTP and LTD in animal brain slices have been established. In the case of LTP, high frequency stimulation (Bliss and Lomo, 1973) (e.g. 100 pulses at 100Hz repeated every 10 seconds) was initially used. However, a more efficient method of LTP induction appears to be with patterned or theta burst stimulation. This pattern of stimulation arose from the observation that neuronal firing in the hippocampus in cats and rats, particularly when exploring novel environments, occurred in bursts of high frequency discharges occurring at the theta frequency (4-7 Hz) (Kandel and Spencer, 1961). It was proposed that this pattern of firing might represent the physiological substrate of learning, and indeed, conditioning paradigms based on theta burst patterns appear to be a reliable method of inducing LTP, and perhaps more powerful than regular high frequency stimulation (Lynch et al., 1977). A typical theta burst pattern is of a burst of 4 pulses at 100Hz delivered every 200ms (i.e at 5 Hz) in a train lasting 10 seconds. This train is usually repeated after a 10 second pause, usually for a total of ten trains of stimulation.

LTD induction is typically achieved via low frequency stimulation at 1-5Hz. This stimulation is given continuously for 20-30 minutes. Interestingly, high frequency stimulation and even theta burst stimulation can induce LTD

providing the stimulation is given for a long enough period and in a continuous, rather than intermittent fashion (Heusler et al., 2000). LTD can also be produced using protocols that would usually cause LTP induction by changing certain qualities of the brain slice, for example hyperpolarising the post-synaptic membrane (Randic et al., 1993).

Over one hundred molecules have been suggested as playing a role in LTP and LTD (Cohen et al., 1991), but it seems most likely that the interaction between glutamate receptors, calcium and AMPA receptors forms the basis of LTP/LTD in excitatory pathways.

Inducing plastic change in human motor cortex.

Repetitive transcranial magnetic stimulation

Transcranial magnetic stimulation is a non-invasive method of inducing an electric current in the brain via the use of a time-varying magnetic field applied to the skull. The magnetic field is delivered through a figure-of-eight or circular coil held on the surface of the skull.

Single pulses of TMS can, when given at the correct intensity over the correct area of cortex, produce a descending volley in the corticospinal pathway and muscle activation. However, when a train of pulses is given, changes can be produced in the stimulated area of cortex that outlast the period of

conditioning. This is the case even if the pulses are delivered at sub-threshold intensities (i.e intensities of stimulation that produce no recordable muscle activation). It is proposed that these changes are analogous to LTD and LTP effects produced in brain slices. Direct recording from synapses pre and post rTMS is not possible, so at the present time one cannot be certain that the observed effects of rTMS are indeed due to LTP/LTD.

A variety of protocols of rTMS have been devised for the induction of long-term changes in cortical excitability. The major limitation of these protocols is that early in the development of rTMS techniques, high frequencies of stimulation (20Hz and above) were found to be capable of inducing seizures in human subjects(Wassermann et al., 1996), and therefore internationally agreed safety guidelines were introduced, restricting the frequency of stimulation that could be used in human subjects(Wassermann, 1998). This is notable given that the typical protocols of LTP induction in animal brain slices use stimulation frequencies of 50-100Hz.

The most commonly used protocol for induction of an LTD-like effect is 1 Hz rTMS delivered at 90% resting motor threshold for 20-30 minutes. With this protocol, reductions in cortical excitability have been measured lasting for 30-40 minutes after the end of conditioning (Chen et al., 1997; Maeda et al., 2000b; Touge et al., 2001). These changes have been demonstrated electrophysiologically by comparing the size of motor evoked potential produced from single pulse cortical stimulation at a set intensity of magnetic

stimulation before and after conditioning. They have also been demonstrated by using positron emission tomography before and after rTMS. A [^{18}F]FDG-PET study showed increased glucose metabolism of bilateral primary motor areas and left SMA after subthreshold 5-Hz rTMS over left primary motor area, indicating that the effects of rTMS are not limited solely to the site of stimulation (Peinemann et al., 2000). Similarly, 1-Hz rTMS over left primary motor cortex resulted in widespread bilateral decreases in rCBF measured with $H_2{}^{15}O$-PET that lasted for at least one hour after stimulation in prefrontal, premotor, primary motor cortex and left putamen (Siebner et al., 2003). A functional MRI (fMRI) study (Lee et al., 2003) confirmed the widespread changes induced by subthreshold 1-Hz rTMS over the primary motor area. The same study also showed that the contralateral premotor area and the unaffected primary sensorimotor area increased their activity to compensate for the suppressive effect of the rTMS.

5Hz rTMS has been used to produce LTP-like effects in human cortex. Due to technical problems with coil overheating, trains of 5Hz stimulation are typically given in an intermittent fashion (Berardelli et al., 1998a; Fierro et al., 2001). This is notable, as continuous 5Hz stimulation protocols are used to induce LTD in animal studies. It seems possible that the intermittent nature of these 5Hz protocols is responsible for the direction of effect noted in human studies.

Higher frequency, higher intensity rTMS has been used, for example a 20 pulse train at 20 Hz and 150% RMT (Pascual-Leone et al., 1994). These studies have, in general, produced short lasting (seconds to minutes) increases in cortical excitability.

Although physiological effects have been noted after rTMS, behavioural effects have been elusive. Most studies confirm that there is no effect of 1 Hz motor cortex rTMS on simple motor tasks, e.g. finger tapping speed or maximal and mean peak force and peak accelerations of finger movements (Chen et al., 1997; Muellbacher et al., 2000). Muellbacher et al. (Muellbacher et al., 2002) also reported that the retention of behavioural improvement, but not the performance of other basic or well practiced motor tasks or recall of the newly acquired motor skill, was disrupted by low frequency rTMS on the motor cortex. More complicated tasks, such as the serial reaction time task (Siebner et al., 1999) may be affected by rTMS. Twenty minutes 1 Hz rTMS on the motor or premotor area subtly slowed the reaction time in a visual cued choice reaction time task (Schlaghecken et al., 2003). A 10 min train of 1 Hz rTMS on the motor cortex improved ipsilateral sequential simple finger movements (Kobayashi et al., 2004).

Interventional Paired Associative Stimulation (IPAS)

It is possible to produce long-lasting changes in the excitability of motor cortical pathways in humans using paired stimulation of a sensory afferent

(via direct stimulation of the nerve) and the homologous cortical efferent (via a single TMS pulse over the correct area of motor cortex). If trains of such stimulation are given, then increases and decreases in cortical excitability can be produced that outlast the period of stimulation by minutes to hours.

The exact timing of the afferent and efferent pulses are important in determining the direction of change in cortical excitability. Sensory stimulation preceeding cortical stimulation by 25ms causes an increase in cortical excitability, whereas a gap of 10ms causes a decrease in cortical excitability (Wolters et al., 2003).

Similar protocols have been used to induce LTP/LTD in animal preparations (Baranyi and Feher, 1981; Hess et al., 1996; Hess and Donoghue, 1994). In humans, the effects of IPAS can be blocked by NMDA antagonists such as dextromethorphan. Such pharmacological studies have not been performed in rTMS, and therefore it is possible that the mechanism of effect of rTMS is different from that of IPAS.

Direct Current Stimulation

It is possible to produce long-lasting changes in the excitability of the motor system through the use of a weak direct current delivered through an electrode placed on the scalp over the motor area (Nitsche and Paulus, 2000). Depending on the polarity of the current, the direction of change in cortical

excitability can be altered. With the positive electrode over the motor area (anodal stimulation), and increase in excitability can be produced. With the negative electrode over the motor area (cathodal stimulation) a decrease in cortical excitability is produced (Nitsche and Paulus, 2000).

Plasticity and dystonia

Converging experimental and clinical evidence would suggest a role for disordered sensorimotor plasticity in individuals with dystonia. Abnormal brain plasticity in dystonia offers an attractive basis for a new hypothesis to explain the pathogenesis of dystonia.

Mapping studies

Indirect mapping of motor and sensory cortices has been performed in dystonia using functional imaging and trascranial magnetic stimulation techniques. In patients with primary dystonia these studies have in general found an enlargement or receptive fields and a blurring of margins such that representations of adjacent digits, for example, tend to overlap (Delmaire et al., 2005; Thickbroom et al., 2003). Abnormal motor maps are not fixed, but can change with effective treatment, for example with botulinum toxin injections (Thickbroom et al., 2003).

rTMS and IPAS

rTMS and IPAS have been used to explore differences in the ability to induce plastic changes in those with dystonia compared to normal subjects.

Quarteronne and colleagues used interventional paired associative stimulation (IPAS) to explore the ability to induce plastic changes in 10 subjects with focal hand dystonia and 10 normal subjects (Quartarone et al., 2003). Low-frequency median nerve stimulation, paired with suprathreshold transcranial magnetic stimulation (TMS) over the optimal site for activation of the abductor pollicis brevis (APB) muscle typically induces a long-lasting increase in the excitability of corticospinal output neurons, if median nerve stimulation is given 25 ms before TMS. Motor evoked potentials (MEPs) were recorded from right APB muscle and right first dorsal interosseus (FDI) muscle. Resting and active motor threshold, mean MEP amplitude at rest, short-latency intracortical inhibition (SICI) at an interstimulus interval of 2 ms and the duration of the cortical silent period (CSP) were assessed immediately before and after IPAS. In both groups, IPAS led to an increase in resting MEP amplitudes which was more pronounced in the right APB muscle. Compared with healthy controls, stimulation-induced facilitation of MEP amplitudes was stronger in patients with writer's cramp. In addition, only patients showed a slight decrease of resting and active motor thresholds after conditioning. It therefore appears that it is "easier" to induce plastic change in those with dystonia compared to normal subjects using IPAS.

Siebner and colleagues used rTMS to explore this similar issue(Siebner et al., 2003). In this experiment subjects with focal hand dystonia and normal controls received 1Hz rTMS or sham stimulation, and then had a PET scan. Widespread changes were seen in the cortex which were greater in subjects with dystonia compared to controls.

A recent study has assessed homeostatic mechanisms of plasticity in dystonia by examining the result of pre-conditioning the motor cortex with either anodal direct current stimulation (which tends to enhance subsequent conditioning with rTMS) or cathodal direct current stimulation (which tends to decrease the effect of subsequent conditioning with rTMS) (Quartarone et al., 2005). Subjects with focal hand dystonia and normal subjects were given these different types of direct current stimulation prior to 1Hz rTMS. As expected, in normal subjects anodal DCS enhanced the inhibitory effects of subsequent 1Hz rTMS while cathodal DCS reversed the effect of 1Hz rTMS and produced facilitation of motor evoked potential. In subjects with focal hand dystonia, no reliable effect of cathodal or anodal DCS was demonstrated interpreted as a failure of homeostatic plasticity mechanisms in dystonia.

Clinical data

Clinical studies have demonstrated that a proportion of individuals who excessively practice a skilled movement pattern, for example professional

musicians (Frucht, 2004), can develop dystonia in the trained limb. This has also been noted in an animal model of dystonia (Byl et al., 1996). Accidental or surgical trauma to a body part, which can enhance long-term potentiation (LTP) in the somatotopic area of cortex, can in certain individuals either induce or worsen pre-existing dystonia (Jankovic, 2001). As further support for the hypothesis of a primary pathological role for excessive plasticity in dystonia, in the therapeutic setting there has been some limited success reported with the use of interventions designed to restore a more normal pattern of synaptic connectivity in the sensori-motor system (e.g. limb immobilisation (Priori et al., 2001), Braille reading (Zeuner and Hallett, 2003), and constraint-induced movement therapy (Candia et al., 1999)).

Conclusion

The data presented above suggest an important role for abnormal plasticity of the sensorimotor system in the genesis of dystonia. An hypothesis that might explain these observations is that in dystonia there is an increased propensity to form associations between inputs and outputs which leads to activation of inappropriate patterns of muscle activity during voluntary movement. It should be noted that an increased susceptibility to undergo changes in synaptic effectiveness (i.e. increased "plasticity") is compatible with other theories of dystonia, such as lack of "surround inhibition" (Mink, 2003; Sohn and Hallett, 2004) or disordered sensory "gating" of movement (Kaji, 2001). Indeed, one can speculate that such a change at the synaptic level could be

the underlying reason why such changes develop in the first instance. Reduced "surround inhibition" would be the consequence of an increased tendency to form excitatory connections within sensori-motor pathways, whereas lack of sensory "gating" would reflect increased associations between sensory inputs and motor outputs that are normally not present in healthy subjects.

Although the response of dystonic subjects to indirect tests of synaptic plasticity (IPAS and rTMS) has previously been found to be more intense and longer-lasting than that of normal subjects (Quartarone et al., 2003; Siebner et al., 2003) it is not clear if this represents a primary abnormality in the control of synaptic plasticity in dystonia. The mere presence of a particular physiological abnormality in dystonic individuals is not sufficient to demonstrate its pathogenicity.

Placing abnormalities in the system that regulates plastic changes within the motor system in the centre of a model to explain primary dystonia provides an attractive hypothesis which is concordant with a wide range of clinical and experimental data. The unique model provided by manifesting and non-manifesting DYT1 gene carriers gives an ideal subject group in which to test this hypothesis.

Chapter 3

Methods

Subject Ascertainment

Manifesting DYT1 mutation carriers were ascertained from a pre-existing database of dystonic patients who tested positive for the DYT1 mutation. These patients were contacted by telephone and the study was discussed with them. In subjects who expressed an interest in the study, and arrangement was made to visit them and as many of their family members as possible in order to perform clinical evaluation, and to ascertain non-manifesting DYT1 mutation carriers.

Family members with dystonia were clinically examined and videoed, according to the video scheme outlined in the paper describing the Burke-Fahn-Marsden dystonia scale. Details on age at onset, precipitants to onset, other medical history, progress of dystonia and response to medication and other treatment was recorded.

Family members without dystonia were asked to give a blood sample for DYT1 mutation analysis on the understanding that no results of the gene anaylsis would be made available to them. Subjects who wished to know their gene test result were refered for genetic counselling to the clinical genetics

service at the National Hospital for Neurology: the process of counselling and delivery and follow-up of these patients was therefore performed separate from the study as part of normal NHS clinical service provision. Subjects were informed that both mutation positive and negative subjects would be invited to take part in the elctrophbysiological studies, and therefore an invitation to take part should not be taken as evidence of mutation carriage.

In practice, many asymptomatic family members knew their mutation status, which simplified the potential ethical dilemmas associated with this type of study.

For a period of time after discovery of the DYT1 mutation the National Hosptial for Neurology provided the only service for DYT1 mutation testing in the UK. Because of this, and the fact that many dystonic patients had been referred from all over the UK for specialist opinions, the patients eligible for enrolment in the study were widely distributed throughout the UK.

Clinical Study – methods

As part of the patient ascertainment described above, a number of patients with dystonia positive for the DYT1 mutation were ascertained who did not fit with classical clinical descriptions of patients with DYT1 dystonia. These subjects were selected and their clinical course characterised in more detail. In addition a review was performed of previously published cases of DYT1

dystonia to try to better characterise the possible phenotypes associated with the DYT1 mutation, and the aspects of the phenotype that were most consistent across published cases. This review was performed by simply entering the terms "DYT1", "torsin A" and "TOR1A" into Pubmed, and looking for any articles that contained clinical details of DYT1 positive patients. These details were then collated.

Electrophysiological studies – Methods

Subjects

Many of the same manifesting and non-manifesting DYT1 positive subjects were used for the three different electrophysiological studies described in this thesis. In addition a group of patients with adult-onset primary cervical dystonia were used for one electophysiological study.

DYT1 subjects were recruited from the pool of patients ascertained in the initial phase of the study described above.

We recruited a total of 10 DYT1 gene carriers with clinical dystonia (MDYT1) from the movement disorder clinics at the National Hospital for Neurology and Neurosurgery. Inclusion criteria were 1) genetic analysis positive for the typical DYT1 mutation, 2) onset of limb dystonia prior to the age of 25 with or without subsequent progression, 3) no other cause for dystonia revealed by

investigation, including imaging and blood tests, 4) no brain, spinal or peripheral nerve surgery for dystonia or other cause in the past, 5) no history of other neurological disease, 6) no use of botulinum toxin in the past 4 months. Subjects were permitted to continue their other medications as normal during the study. Clinical details of these patients are given in table 3.1. All patients had clinical dystonia affecting the arm and hand used for electrophysiological testing. A total of eight DYT1 gene carriers without clinical symptoms (NMDYT1) were ascertained by genetic and clinical assessment of family members of the MDYT1 group. Inclusion criteria were 1) genetic analysis positive for the typical DYT1 mutation, 2) clinical absence of dystonia confirmed by personal independent assessment of each patient by two clinicians, 3) no brain, spinal or peripheral nerve surgery for any cause in the past, 4) no history of neurological disease, 5) age over 30. Thirteen healthy controls were recruited from a departmental register of volunteers and from DYT1 mutation negative family members of DYT1 positive subjects. The average age of those in the MDYT1 group was 49 (SD: 9), in the NMDYT1 group was 50 (SD: 8), and in the control group was 42 (SD:7). For one set of experiments, we recruited 6 subjects with adult-onset focal dystonia affecting the neck (torticollis). The inclusion criteria for this group were (i) focal dystonia affecting the neck, with no other neurological disorder; (ii) onset age over 40 years; (iii) no brain, spinal or peripheral nerve surgery for any cause in the past; (iv) no use of botulinum toxin in the previous 4 months.

Table 3.1: Clinical details of the manifesting DYT1 positive subjects.

Subject	Age of Onset	Site of Onset	Current distribution of dystonia	BFM score	Medication	Experiments Completed
1, Male	12	R arm	Generalised	46	None	1 (SP, RI)
2, Female	11	R hand	Segmental	12	Clonazepam, benzhexol	1 (ICI, SP, RI), 2, 3.
3, Female	10	L foot	Generalised	44	Benzhexol	1 (ICI, SP, RI), 2, 3.
4, Male	6	L foot	Generalised	74	Diazepam	1 (SP)
5, Female	3	L foot	Segmental	16	None	1 (ICI, SP, RI)
6, Male	10	R hand	Multifocal	28	None	1 (ICI, SP, RI), 2, 3
7, Female	13	R arm	Segmental	18	Levodopa	1 (ICI, SP, RI), 2, 3
8, Male	12	R hand	Focal	6	Benzhexol	1 (ICI, SP, RI), 2, 3
9, Male	9	R arm	Segmental	6	None	1 (ICI, SP, RI), 2, 3
10, Male	18	L leg	Generalised	27	None	1 (ICI, SP)
11, Female	7	R hand	Focal	12	None	2, 3
12, Male	9	R hand	Segmental	22	None	2, 3

BFM score = Burke, Fahn, Marsden Rating scale score(Burke et al., 1985) , Experiments completed refers to the experiments which the subjects took part in (see below).

Electrophysiological Techniques

Different electrophysiological techniques were used in each of the three main experiments:

1. **Experiment 1 (Chapter 5)** Intracortical inhibition, silent period and reciprocal inhibition in normal subjects, manifesting and non-manifesting DYT1 subjects
2. **Experiment 2 (Chapter 6)** Reciprocal inhibition pre and post 1Hz repetitive transcranial magnetic stimulation in normal subjects, manifesting and non-manifesting DYT1 subjects.
3. **Experiment 3 (Chapter 7)** Measurement of the time course of motor evoked potential changes after continuous theta burst stimulation (cTBS) in normal subjects, manifesting and non-manifesting DYT1 subjects.

These methods are described in turn below.

Experiment 1

Assessments of intracortical inhibition and facilitation (ICI/ICF), cortical silent period (SP) and reciprocal inhibition (RI) were attempted in 10 MDYT1 subjects, 7 NMDYT1 subjects and 13 normal controls. The assessments were all performed on the same day with ICI/ICF and SP in one session, and then RI in a second session.

Intracortical Inhibition and Facilitation.

The technique of ICI measures the influence of a sub-threshold "conditioning" pulse of transcranial magnetic stimulation (TMS) given over the hand motor area on a subsequent supra-threshold "test" pulse given over the same area. Experiments in normal subjects have shown that at short interstimulus intervals (0-4ms) there is a reduction in the size of the MEP elicited from the contralateral first dorsal interosseus (intracortical inhibition)(Kujirai et al., 1993). At interstimulus intervals of between 7 and 15ms there tends to be an increase in the size of the MEP elicited by the supra-threshold stimulus (intracortical facilitation)(Kujirai et al., 1993).

Subjects were seated in a comfortable chair. EMGs were recorded from the right first dorsal interosseous (FDI) using Ag-AgCl electrodes. EMG activity was recorded with a gain of 1000 and 5000. Magnetic stimulation was given using a hand-held figure of eight coil connected though a Bistim module (Magstim Company, UK) to two magnetic stimulators (Magstim Company, UK).

The location of the hand motor area was defined by the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral FDI when the subject was relaxed (the "motor hot-spot"). We defined the resting motor threshold as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 50μV in five out of ten trials.

We defined the active motor threshold as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 200μV in five out of ten trials during a voluntary contraction of the contralateral FDI.

The conditioning stimulus was set at 80% of active threshold. The test stimulus was set at the intensity of magnetic stimulation required to consistently produce an MEP of 1mV.

Subjects received in a random order either the test stimulus alone, or conditioning-test stimuli at interstimulus intervals of 2, 3, 4, 5, 6, 7, 10 and 15ms. Subjects received the stimuli in two blocks of 50 stimuli each. All trials in which EMG movement artefact occurred were rejected online, and that stimulus condition was repeated.

Silent Period

The SP is a period of EMG silence that occurs in a voluntarily contracted muscle following a suprathreshold magnetic stimulation given over the contralateral representative motor area. In normal subjects the SP is typically 120ms, although this can be longer if the stimulation intensity is raised(Inghilleri et al., 1993).

EMGs were recorded as described above. A single magnetic stimulation unit (Magstim Company, UK) was used to deliver the magnetic pulse through a

standard figure of eight coil. Motor thresholds were obtained as described above.

Subjects were asked to squeeze a 2.5cm block between their thumb and index finger. Visual feedback on the intensity of muscle contraction was provided to the subjects, and they were instructed to maintain a constant muscle contraction at about 30% of maximum.

Magnetic stimulation was applied over the contralateral hand motor area at 120% of rest threshold. Twelve stimulations were recorded for each subject. The SP was calculated by measuring the time from the end of the MEP to the reappearance of EMG activity in excess of 20μV. Those trials where voluntary muscle activation exceeded or was less than 30% of maximum were rejected online, and the stimulus was given again.

Reciprocal Inhibition

RI assesses the interaction between stimulation of the radial nerve supplying the extensor muscles of the forearm and the H reflex produced by stimulation of the median nerve. At particular inter-stimulus intervals, a reduction in the size of the H reflex occurs in normal subjects(Day et al., 1984). We grouped these interstimulus intervals into three phases of RI, one occurring at 0ms, one at 10-20ms and one at 70-750ms.

We attached Ag-AgCl electrodes to extensor digitorum communis, and to flexor carpi radialis. Electric pulses were supplied by two constant current generators (Digitimer, UK). One electrical stimulator was used to stimulate the median nerve in the antecubital fossa. Stimulation duration was 1000μs, and the intensity used was that which produced the maximum size of the H reflex. The second electrical stimulator was used to stimulate the radial nerve above the elbow. The duration of the stimulus was 500μs, and the intensity used was that which produced a EMG response of greater than 50μV from extensor digitorum communis.

We recorded H reflex size during stimulation of the median nerve alone, and for interstimulus intervals of –1, 0, 3, 5, 10, 20, 30, 50, 70, 100, 300, 500 and 750 ms. Stimuli were given in a random order in one block of 60 trials and two blocks of 50 trials. Any trials where EMG movement artefact occurred were rejected online, and were repeated.

Statistical Analysis

To assess ICI and ICF, repeated-measures analysis of variance (ANOVA) was used. Because inhibition and facilitation at particular interstimulus intervals have different mechanisms, we grouped means at an "inhibitory" interval (average of 2, 3, and 4ms interstimulus intervals), an "intermediate" interval (average of 5 and 6ms interstimulus intervals), and a "facilitatory" interval (average of 7, 10 and 15ms interstimulus intervals).

To assess SP, one-way analysis of variance was used to compare the three groups.

To assess RI, a two-way ANOVA was used to compare the data between the three groups at each of three interstimulus intervals: "first phase" (interstimulus interval of 0ms), "second phase" (average of interstimulus intervals 10 and 20ms) and "third phase" (average of interstimulus intervals 70-750ms).

Not all subjects were able to participate in all the experiments. Subjects 4 and 10 had no consistent H reflex, and therefore reciprocal inhibition (RI) could not be assessed in them. In subjects 1 and 4, assessments of ICI/ICF were confounded by movement artefact. One subject in the NMDYT1 group also did not have a consistent H reflex, and therefore could not have RI assessed. Statistics were performed using SPSS for Windows 10.0.

Experiment 2

Eight MDYT1 subjects (see table 3.1), 6 NMDYT1 subjects and ten healthy controls were recruited for this experiment.

Reciprocal inhibition (RI) was recorded from subjects using the same method as described above. Following this assessment of RI, 1Hz rTMS was given

over the pre-motor area. The pre-motor area was defined in relation to the motor hand area, defined as the location on the scalp where magnetic stimulation reproduced the largest MEP from the contralateral first dorsal interosseous (FDI) when the subject was relaxed (the "motor hot-spot"). We defined the resting motor threshold as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 50μV in five out of ten trials. The pre-motor area was defined as an area 2.5cm anterior to the motor hot spot. RTMS was administered using a flat figure-of-eight-shaped magnetic coil (outer diameter of each wing: 9.5 cm). The coil was held tangentially to the skull with the handle pointing backward and laterally at a 45 degree angle to the sagital plane.

1200 pulses of rTMS at 1Hz were delivered to the pre-motor area at an intensity of 90% of resting motor threshold. Stimulation was provided by a Magstim rapid stimulator connected to four booster modules (Magstim Company, UK). The pulse waveform was bi-phasic.

Following the period of rTMS, RI was immediately re-assessed using the paradigm described above.

Statistical Analysis

Statistics were performed on grouped data from the whole time course of RI: the first phase of inhibition was defined as inhibition at ISI = 0 ms; the second phase as ISIs = 10, 20 ms; the third phase of inhibition was defined

as the mean inhibition over ISIs = 75 -500 ms. We took the a priori view that the three phases of RI were due to different mechanisms, and therefore performed a two way ANOVA with GROUP (patients vs. controls) and TIME (before vs. after rTMS) as main factors. This two-way analysis was followed by paired t tests to probe the nature of any interaction. Statistics were performed using SPSS for Windows 10.0.

Experiment 3

Eight MDYT1 subjects, 6 NMDYT1 subjects, 7 subjects with adult onset cervical dystonia and 7 healthy control subjects were recruited.

Subjects were seated in a comfortable chair. Ag-Cl electrodes were attached to the dominant first dorsal interosseous muscle (FDI) (the right hand in all subjects) using a belly-tendon montage. EMG signals were amplified using a gain of 1000 and 5000 via a Digitmer amplifier (Digitimer, UK).

We identified the "motor hot-spot" relating to the dominant FDI using a single magnetic stimulator (Magstim 200, Magstim Company, Dwyfed, UK) connected to a hand-held figure of eight coil with an outer winding diameter of 70mm. The coil was held with the handle pointing in the anterior-posterior plane, which is thought to preferentially activate neurons transynaptically. The motor hotspot for the FDI was defined as the area on the scalp where a magnetic stimulus of a set intensity produced the largest size of MEP from the

contralateral FDI. This spot was marked on the scalp. The intensity of the magnetic stimulus was adjusted to produce a MEP of approximately 1mV for each subject, and this intensity was used for all assessments of MEP size for the rest of the experiment.

The active motor threshold (AMT) was calculated as the minimum intensity of magnetic stimulation given over the motor hotspot capable of producing an MEP of greater than 200μV in 5 out of 10 trials while subjects were performing a voluntary contraction of FDI at about 20-30% of maximum. A constant level of muscle contraction was achieved by the use of visual feedback.

rTMS

Repetitive transcranial magnetic stimulation (rTMS) was delivered using the same figure of eight coil described above connected to a Rapid-stim machine (Magstim Company, Dwyfed, UK) and four booster modules. We used a novel paradigm of rTMS based on theta burst patterns (see *Appendix 1* for a full description of this technique). The basic theta burst pattern used was a train of three 50Hz pulses given every 200ms (i.e. at 5Hz). This pattern was given in a continuous fashion for 20 seconds (a total of 300 pulses). The intensity of this stimulation was 80% of AMT. We have previously found this pattern of stimulation capable of producing consistent MEP suppression for about 20 minutes following the end of stimulation.

For each subject we measured MEP size in response to single pulse stimulation delivered at the set intensity described above. 30 MEPs were recorded in this fashion, and the resulting mean MEP size defined the baseline excitability of the hand motor area.

rTMS was then delivered according to the paradigm described above. Immediately after the end of rTMS, and at five minute intervals after this, a block of 15 MEPs was recorded by stimulation of the hand motor area using the same intensity of stimulation used in the baseline assessment of MEP. Recording was continued every five minutes until 35 minutes after rTMS.

Mean MEP size was calculated for each subject for each five minute interval after rTMS. MEPs were normalised with respect to baseline MEP size. Group differences in change in MEP size following rTMS were assessed using a 2 way ANOVA with TIME (time following rTMS) and GROUP as main factors. Independent sample t-tests were used for post-hoc comparisons. Statistical analysis was performed using SPSS for Windows version 11.0.

Chapter 4

Clinical data obtained during ascertainment of patients for electrophysiological studies.

During patient ascertainment for this study, a number of families with DYT1 dystonia were reviewed, and we identified a number of patients with phenotypes in DYT1 positive individuals that did not fit with the "typical" phenotype said to be associated with the mutation. Example case histories of five such patients are given below. This prompted us to analyse all previously reported patients with DYT1 dystonia with regard to clinical presentation and course, in order to better characterise the range of phenotypes associated with the DYT1 mutation.

Case 1: Late Presentation

This 71-year-old man was of Ashkenazi Jewish origin, and had a normal birth and early development. He was well until his mid-thirties, and worked in the financial sector at a job that required a significant amount of writing by hand. At the age of 35 he noticed for the first time that his writing had deteriorated due to the onset of tremor in the right hand. Over the next two years, the tremor spread to involve the left hand also. These symptoms were stable until the age of 69 when he noticed a worsening of the tremor in his left hand so

that he was unable to perform routine tasks with that hand. Family history was positive for bilateral arm dystonia in his son, also with late onset in his thirties. His son was positive for the DYT1 mutation.

Examination at the age of 71 revealed a clear rest tremor of both arms. The tremor was more severe on posture, and was coarse and proximal in nature. When attempting to write he had an abnormal grip with aggravation of the tremor. There was mild dystonic posturing of the left hand when the arms were outstretched. Since there had been late worsening of the tremor, the diagnosis of Parkinson's disease was considered. However, a Dopamine Transporter scan (DAT SPECT) was normal.

Case 2: Late presentation triggered by injury

This 68-year-old woman was entirely well until the age of 38, working in a department store and bringing up a family. She had never noticed any difficulty in walking. At the age of 38 she tripped and twisted her left ankle. Within a few days following this injury she developed inversion and plantar flexion of the left foot on walking that has persisted to the present day. No other symptoms emerged until the age of 60 when she noticed that her voice had become hoarse.

Her sister and her nephew both had early-onset dystonia, and were positive for the DYT1 gene abnormality. Her father was said to have had "weak ankles" and wore a splint on his left foot.

Examination revealed a clear dystonic posturing of the left foot on walking. In addition, her speech was dystonic with a harsh voice and inaccurate articulation of certain sounds. The rest of the examination was normal.

Case 3: Late Progression.

This 65-year-old right-handed woman is the sister of Case 2 above. After a normal early development, she developed inversion of the left foot at the age of five. She had no problem with her general functioning apart from a slightly unusual gait.

At the age of 59 she began to develop problems with writing. Although she had previously been noted to have some dystonic posturing of the right arm that manifested when walking, her right hand had not been affected and she had been able to write without difficulty all her life. At the age of 59 for the first time she noticed that her hand began to adopt an unusual posture during writing, and that the quality of her handwriting had deteriorated, so that she was prompted to seek medical attention.

On examination she had tremulous dystonia of the left foot at rest and on walking. The right leg was mildly affected. She had a mild postural tremor of the right arm, and clear dystonic posturing of the right hand during writing.

Case 4: Onset of Dystonia after Drugs.

This 67 year old woman was well until the age of 47. She then developed problems with low mood and suffered episodes of mania. Manic depression was diagnosed, and after initial treatment with benzodiazepines, haloperidol was introduced. One year later, when she was 50, she noticed that her head was turning involuntarily to the left while she was walking. Over the next year she noticed the gradual onset of abnormal posturing of her right foot and spine when walking. In addition, the quality of her voice changed with her articulation becoming less clear. Her symptoms then stabilised, and have not changed since.

Her family history was positive for early onset dystonia in two of her three children, both of whom were positive for the DYT1 mutation. Her son developed limb dystonia at the age of seven with subsequent generalisation, including spread to craniocervical and bulbar muscles. Her daughter developed dystonia in the right hand at the age of 18, manifesting mainly on writing and playing the trumpet. She is now 25 and has developed no further symptoms. There was no family history of dystonia in the parents or grandparents of case 4.

Examination revealed torticollis to the left, exacerbated by walking, as well as mild right foot dystonia and moderate axial dystonia. She had dystonic involvement of her speech.

Case 5: Severe Bulbar involvement

This 19 year old man was well until he developed dystonic posturing of the right arm at the age of seven. The dystonia became generalised within one year and was severe involving all four limbs and with a very marked axial dystonia producing retrocollis and an opisthotonic posture. His dystonia proved refractory to treatment with benzhexol, tetrabenazine, L-dopa, clonazepam and baclofen.

At the age of 13, he developed progressive bulbar involvement with dysarthria and problems with swallowing. These problems became severe, and resulted in frequent chest infections secondary to aspiration. A gastrostomy tube was inserted when he was 17 years old as swallowing even thickened fluids was unsafe.

Due to his severe bulbar involvement, secondary dystonia was suspected and he was investigated accordingly. However, all tests were normal, including MR imaging of his brain. When DYT1 testing became available, he was found to be positive for the typical GAG deletion. There was no family history of a similar disorder.

Bilateral globus pallidus internus stimulators were inserted in 2001. He has made a significant improvement since this time, so that he is now swallowing and speaking well, and has noted a marked reduction in limb dystonia.

Review of previously published cases with DYT1 dystonia

Prompted by these unusual cases, we undertook a review of previously published cases with the DYT1 mutation (table 1.3) The number of cases reported to date is small (219 cases), and some of these cases may have been reported simply because they are unusual, perhaps biasing the cases in the literature towards those that are atypical (Brassat et al., 2000; Bressman et al., 2000; Gasser et al., 1998; Gatto et al., 2003; Ikeuchi et al., 1999; Im et al., 2004; Kabakci et al., 2004; Kamm et al., 1999; Kamm et al., 2000; Lebre et al., 1999; Leube et al., 1999; Leung et al., 2001; Major et al., 2001; Matsumoto et al., 2001; Nomura et al., 2000; Opal et al., 2002; Slominsky et al., 1999; Valente et al., 1998; Wong et al., 2005). However, studies with larger numbers of unselected patients such as those by Bressman (Bressman et al., 2000), Lebre (Lebre et al., 1999) ,Slominsky (Slominsky et al., 1999) and Valente (Valente et al., 1998), show how variable the phenotype can be. However, amongst this variability, two clinical features stand out in their relative constancy: 1) onset of symptoms before the mid-twenties, and 2) onset of dystonia in a limb. Age of onset of dystonia in the reported cases ranges from 4 months (Nomura et al., 2000) to 64 years of age (Opal et al., 2002). However, despite this large range, onset after the mid twenties is rare

(only 20 out of 219 reported cases (9%)), and in most the onset is in late childhood or early teens. Onset of dystonia almost always occurs in a limb, although four cases with onset in the neck (Bressman et al., 2000), one case with onset in the larynx (Bressman et al., 2000) and one case with onset in the trunk (Ikeuchi et al., 1999) have been reported. Overall, onset appears to be just as likely to occur in the upper limb as the lower limb, although in the unselected largely English cohort reported by Valente (Valente et al., 1998) only 14% of patients had onset in the leg. The impression that non-Jewish DYT1 positive individuals are more likely to have onset in the arm than the leg is not supported by Bressman's large sample (Bressman et al., 2000). In this study, a comparison of site of onset between Jewish (n=52) and non-Jewish (n=45) individuals, showed that 37% Jewish individuals had onset in the leg, compared to 60% of non-Jewish individuals.

Thus, most of the variability seen in the reported cases of DYT1 dystonia is actually in the clinical pattern (distribution) of the dystonia. Although progression to generalised dystonia appears to be the norm, from our review the number of cases remaining with focal dystonia after prolonged follow-up is striking (21% of Bressman's sample), as is the frequency of cranio-cervical involvement (9-40% in the larger unselected studies). The relatively low frequency of progression to generalised dystonia in Bressman's study (57%) may reflect a more stringent application of the clinical categorisation of dystonia compared to other studies, as 10% of their sample are reported to have multifocal dystonia, and 12% segmental dystonia.

Table 3.1: Summary of previously published cases of DYT1 dystonia

Study	No of Patients	Mean age at onset in years (SD)	Site of onset % (n)	Progression % (n)	Mean length of follow up (SD)	Cranial Involvement? % (n)
Wong (2005)	1	4	Leg	Generalised	6	No
Im (2004)	5	13 (5) Range 7-20	Arm: 40% (2) Leg: 40% (2) Neck 20% (1)	Generalised: 60% (3) Segmental: 20% (1) Focal: 20% (1)	Not stated	Yes: 40% (2) No: 60% (3)
Kabakci (2004)	5	13 (10.2) Range 2-31	Arm : 60% (3) Leg: 40% (2)	Generalised: 80% (4) Segmental: 20% (1)	Not stated	Yes: 60% (3) No: 40% (2)
Gatto (2003)	1	9	Arm	Segmental	37	No
Matsumoto (2001)	6	13.7 (10.5) Range 9-35	Arm: 66.6% (4) Leg: 33.3% (2)	Generalised: 50% (3) Focal: 50% (3)	14.2 (18.2) Range 1-49	Not Stated
Major (2001)	3	13.3 (3.5) Range 10-17	Arm: 66.6% (2) Leg: 33.3% (1)	Generalised:66.6% (2) Segmental: 33.3% (1)	24 (15.3) Range: 7-37 yrs	Yes: 66.6% (2) No: 33.3% (1)
Bressman (2000)	97	14 (9) Range: 4-44	Leg: 47.4% (46) Arm: 48.5% (47) Neck: 3.1% (3) Larynx: 1.0% (1)	Generalised:56.7%(55) Multifocal: 10.3% (10) Segmental: 12.4%(12) Focal: 20.6%(20)	25yrs (15.7) Range not stated	Yes: 9.3% (9) No: 90.7% (88)
Nomura (2000)	10	11.1 (6.1) Range 0.33-18	Leg: 20% (2) Arm: 80% (8)	Generalised: 50% (5) Segmental: 10% (1) Focal: 40% (4)	22 (13.9) Range: 7-48	Yes: 20% (2) No: 80% (8)
Brassat (2000)	5	8.6 (3.04) Range: 6-12	Leg: 40% (2) Arm: 60% (3)	Generalised: 80% (4) Segmental: 20% (1)	22.5 (18) Range: 1.5-45	Yes: 80% (4) No: 10% (1)
Lebre (1999)	10	9 (4) Range: 5-20	Arm: 90% (9) Leg: 10% (1)	Generalised: 70% (7) Segmental: 20% (2) Focal: 10% (1)	22 (17) Range: 3-45 yrs	Yes: 40% (4) No: 60% (6)
Leube (1999)	3	12.7 (4) Range 9-17	Leg: 66.6% (2) Arm: 33.3% (1)	Generalised:66.6% (2) Focal: 33.3% (1)	Not Stated	Yes: 33.3% (1) No: 66.6% (2)
Slominsky (1999)	24	Not Stated	Not Stated	Generalised: 92%(22) Focal: 8% (2)	Not Stated	Not Stated. One patient with stuttering only.
Kamm (1999)	4	9.8 (5.1) Range: 5-17	Leg: 50% (2) Arm: 50% (2)	Generalised: 75% (3) Multifocal: 25% (1)	14.3 (10.6) Range: 2-25	Yes: 0% (0) No: 100% (4)
Ikeuchi (1999)	1	13	Shoulder/Trunk	Axial dystonia	12	Yes
Valente (1998)	22	9.9 (4.3) 2-21	Leg: 86.3% (19) Arm:13.6% (3)	Generalised:86.3%(19) Segmental: 9% (2) Focal: 4.5% (1)	Not Stated	Yes: 29% (4) No: 79% (18)
Gasser (1998)	5	14.9 (10.9) Range: 9-38	Leg: 0% Arm:100% (5)	Focal: 80% (4) Unknown: 20% (1)	22.5 (7.9) Range: 12-28	Yes: 0% (0) No: 100% (5)

Guidelines relating to the testing of patients with dystonia for the DYT1 gene have been proposed (Bressman et al., 2000). These guidelines were based on DYT1 testing in a cohort of 267 individuals with primary torsion dystonia, 168 of whom were of Ashkenazi Jewish descent. Notable differences in the ability of clinical characteristics to predict DYT1 positivity were observed between Jewish and non-Jewish individuals. When using age of onset of dystonia before the age of twenty-six as the sole criterion for DYT1 testing, specificity in the non-Jewish cohort was only 43%, compared to 63% in the Jewish cohort. Using "onset in a limb" as the sole criterion for testing gave a specificity in the non-Jewish cohort of only 56%, compared to 81% in the Jewish cohort. Combination of these two factors gave a specificity of 69% for the non-Jewish cohort and 88% for the Jewish cohort. This would argue for a modification of the guidelines for DYT1 testing in non-Jewish individuals with dystonia. Age of onset prior to the age of 26 is probably not a sufficient factor on its own to guide DYT1 testing in non-Jewish individuals, and should be combined with onset of dystonia in a limb. This reflects the lower gene frequency of the DYT1 mutation in the non-Jewish population. In fact, the DYT1 mutation may not be the commonest cause of early-onset generalised dystonia in the non-Jewish population. A recent study of presumably non-Jewish children with limb-onset dystonia with an average age of onset of 8 found only 5 out of 30 (17%) to carry the GAG deletion (Zorzi et al., 2002).

We can apply the above guidelines to the 5 individuals reported here who had unusual phenotypic presentation of dystonias associated with the DYT1 mutation. Case 1 had onset of dystonic (initially task-specific) tremor in his thirties but with clear worsening much later in life, which was the unusual aspect. In fact, it was wondered whether he might have incidental Parkinison's disease, but a DAT SPECT scan was normal. Despite the late worsening of his symptoms, the onset of the symptoms was in a limb, and the phenotype resembled that seen in primary dystonia, suggesting that the clinical syndrome in this patient was due to the DYT1 mutation rather than another cause.

Case 2 had onset of dystonia in the foot following trauma, with later involvement of the larynx. There has been considerable debate concerning the role that environmental factors (particularly trauma) might play in triggering symptoms in other forms of primary dystonia, but little is known about their role in the onset of dystonia in DYT1 gene carriers. A report of monozygotic twins with familial adult-onset craniocervical dystonia suggested that trauma might have played a role in the greater severity of dystonia in one of the twins (Albanese et al., 2000). Epidemiological studies of patients with blepharospasm have implicated facial trauma as a risk factor for the development of the condition (Defazio et al., 1999). The triggering of primary dystonia by trauma should be differentiated from acute post-traumatic dystonia, which leads to a relatively fixed dystonia, the absence of sensory gestes and a poor response to botulinum toxin (Goldman and Ahlskog, 1993;

Tarsy, 1998). In case 2, there was a clear temporal relationship between peripheral trauma and onset of dystonia in the injured limb. The dystonia affecting the foot was not of a fixed type, and was only noticeable during walking. Some years later she developed evidence of laryngeal dystonia. This clinical presentation therefore suggests that her dystonia was primary dystonia due to the DYT1 mutation, triggered by injury, rather than acute-post traumatic dystonia with coincidental DYT1 positivity. This is interesting, as it might suggest a role for environmental factors in the phenotypic penetrance of the DYT1 gene.

Although case 3 had a typical onset of DYT1 dystonia, she developed late progression of dystonia in the right hand, which is unusual. Similarly to case 1, the dystonia progressed in a limb with a phenotype similar to that seen in primary dystonia, suggesting a causal role for the DYT1 mutation in this case. There was no apparent triggering factor for the progression of the dystonia.

In case 4, there was a temporal relationship between neuroleptic drug use and the onset of dystonia. It could be argued that the observed clinical phenotype was due to tardive dystonia, and that the DYT1 mutation was not pathogenic. This argument is supported by the site of onset of the dystonia (the neck), and the similarity of the phenotype to that seen in tardive dystonia (mainly axial involvement). However, it is possible that the presence of the DYT1 gene in this patient increased her susceptibility to developing

dystonia following neuroleptic drug use. The true pathogenesis of the dystonic syndrome in this case therefore remains unclear.

Our fifth case had bulbar involvement, severe enough for PEG insertion to be required, early on in the course of progressive dystonic symptoms. He otherwise had a typical presentation of DYT1 dystonia with onset of dystonia in a limb below the age of 26, with subsequent generalisation. Early bulbar involvement is usually a "red flag" to consider secondary dystonia, but this case demonstrates that DYT1 dystonia can occasionally also manifest with severe bulbar involvement.

The low phenotypic penetrance of DYT1 dystonia creates a diagnostic difficulty in patients with atypical clinical presentations who are positive for the DYT1 mutation. Some such patients may in fact be true asymptomatic carriers of the DYT1 gene, and simply have another unrelated cause for their clinical syndromes. For example, a patient with a psychogenic movement disorder in association with the DYT1 mutation has been reported (Bentivoglio et al., 2002). If more were known about the genetic and/or environmental determinants of penetrance in DYT1 dystonia then it would be possible to determine in which of these atypical clinical cases the DYT1 mutation was pathogenic. At the present time, however, it seems sensible that the onset of dystonia in a limb, and the similarity of the clinical phenotype to that seen in primary dystonia are useful factors that point towards the clinical syndrome being a true manifestation of the DYT1 mutation.

In general, therefore, the guidelines for DYT1 testing proposed by Bressman (Bressman et al., 2000) seem reasonable. For non-Jewish individuals, the addition of "limb onset" to age of onset would be likely to improve the specificity of the guidelines. A positive family history of early-onset dystonia should swing the balance towards DYT1 testing in family members with late-onset dystonia or other atypical presentations. However, as cautioned above, DYT1 positivity in such individuals does not necessarily imply a causal relationship between the observed clinical syndrome and the DYT1 mutation. Limb onset of dystonia and a phenotype typical of primary dystonia provide supporting evidence for such a relationship.

This review of reported cases of DYT1 dystonia does *not* answer some important questions raised by the five unusual cases that we have presented. Does late progression of symptoms occur in other cases of DYT1 dystonia, and if so, how common is this? This is important in counselling patients with DYT1 dystonia about their likely prognosis. Are environmental triggering factors such as trauma or drugs important in the genesis of dystonic symptoms? If so, then there are implications for asymptomatic gene carriers to, if possible, avoid exposure to such triggers. Further detailed studies assessing the clinical characteristics of manifesting and non-manifesting DYT1 positive individuals will help to define the full range of the DYT1 phenotype, and will therefore aid diagnostic and gene testing decisions in the future.

Chapter 5

Cortical, brainstem and spinal inhibition in DYT1 mutation carriers.

The following experiments address the hypotheses outlined in chapter 1:

1. That manifesting gene carriers would have similar abnormalities in cortical and spinal motor inhibitory function as previously described in non-genetically characterised primary dystonia.

2. That non-manifesting gene carriers do not develop symptoms as the DYT1 mutation has no consequences for them (perhaps as it is inactivated by some mechanism).

3. That electrophysiological abnormalities are present in non-manifesting gene carriers that affect similar systems to those seen in manifesting gene carriers, but are of a lesser severity, and do not reach the threshold for clinical symptoms to be produced.

4. That non-manifesting gene carriers have only a sub-set of the pathophysiological abnormalities present in manifesting gene carriers, and these are not sufficient to produce clinical symptoms.

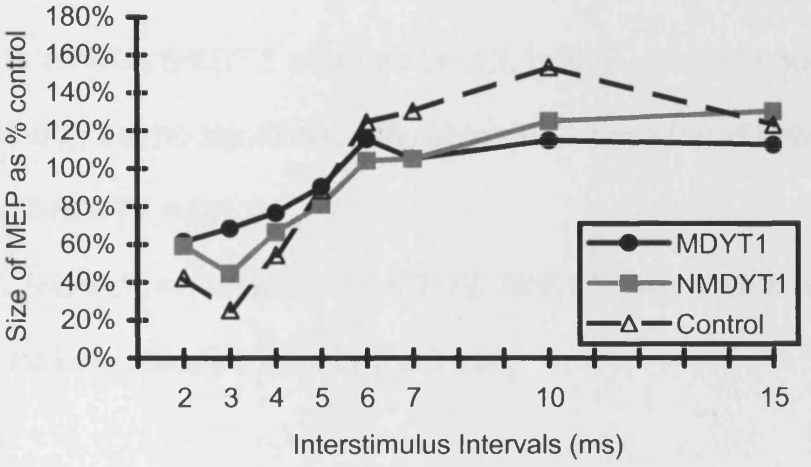
Results

Intracortical Inhibition and Facilitation (ICI and ICF)

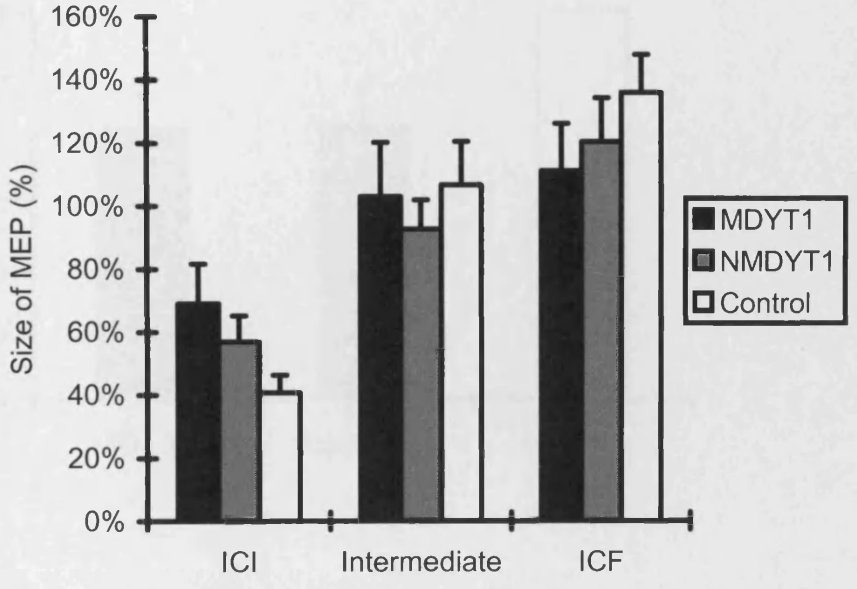
ICI/ICF was compared in 8 MDYT1, 7 NMDYT1 and 8 control subjects. The complete time course at all interstimulus intervals (ISI) is shown in figure 3.1a with grouped data (inhibitory, intermediate and facilitatory ISIs) in figure 3.1b. Repeated measures ANOVA was performed on grouped data with group (MDYT1, NMDYT1 and controls) and ISI (inhibitiory, intermediate and facilitatory) as main factors. As expected, ANOVA showed a highly significant effect of ISI [$F(2,40)=68$, $p<0.001$], but there was also a significant interaction between group and ISI [$F(4,38)=3.5$, $p<0.05$]. Post hoc analysis showed that there was significantly less inhibition in MDYT1 and NMDYT1 subjects compared to controls in the inhibitory interval [$F(1,13)=6.8$, $p<0.05$ and $F(1,13)=5.7$, $p<0.05$ respectively]. There were no significant differences found at the inhibitory interval between MDYT1 and NMDYT1 subjects. No significant differences were found between controls and either group of subjects at the intermediate or facilitatory intervals.

Figure 5.1a and b: Intracortical inhibition and facilitation for MDYT1, NMDYT1 and control subjects. Figure 5.1a shows the size of MEP as a percentage of the unconditioned size at all interstimulus intervals. Figure 5.1b shows the mean size of MEP as a percentage of the unconditioned size at the inhibitiory, intermediate and facilitatory intervals. Error bars represent standard error of the mean.

A



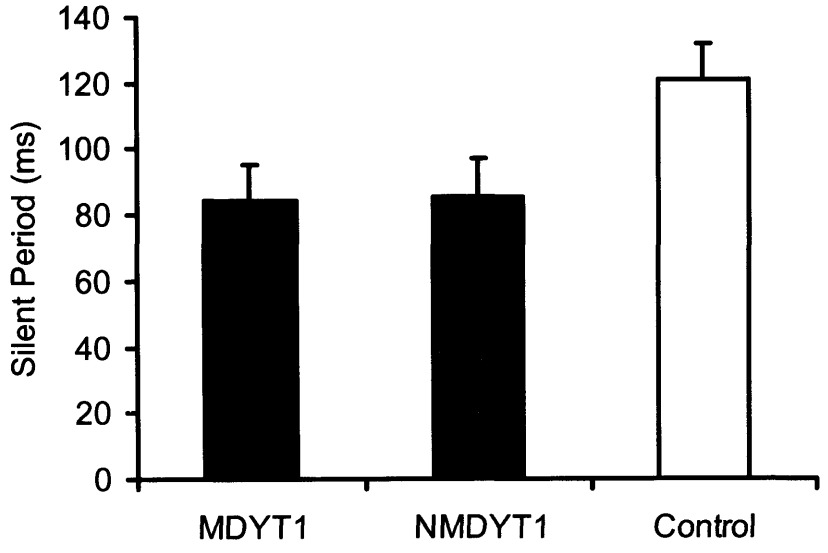
B



Cortical Silent Period (SP)

SP was assessed in 10 MDYT1, 6 NMDYT1 and 8 control subjects. Results are shown in figure 3.2. Oneway ANOVA was performed on the data, and demonstrated a significant effect of group on the length of the silent period [$F(2,21)=3.9$, $p<0.05$]. Post hoc analysis using independent sample t tests was then performed. The SP was shorter in both groups of gene carriers compared to controls (MDYT1 subjects: $t=-2.3$, $p<0.05$; NMDYT1 subjects: $t=-2.5$, $p<0.05$), but no significant differences in SP were found between MDYT1 and NMDYT1 subjects.

Figure 5.2: Silent period duration for MDYT1, NMDYT1 and control subjects. Error bars indicate standard error of the mean.

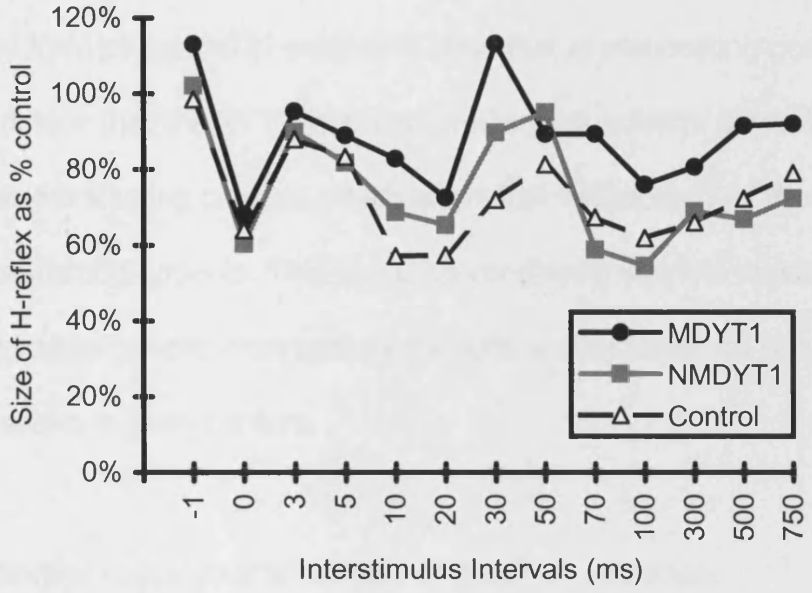


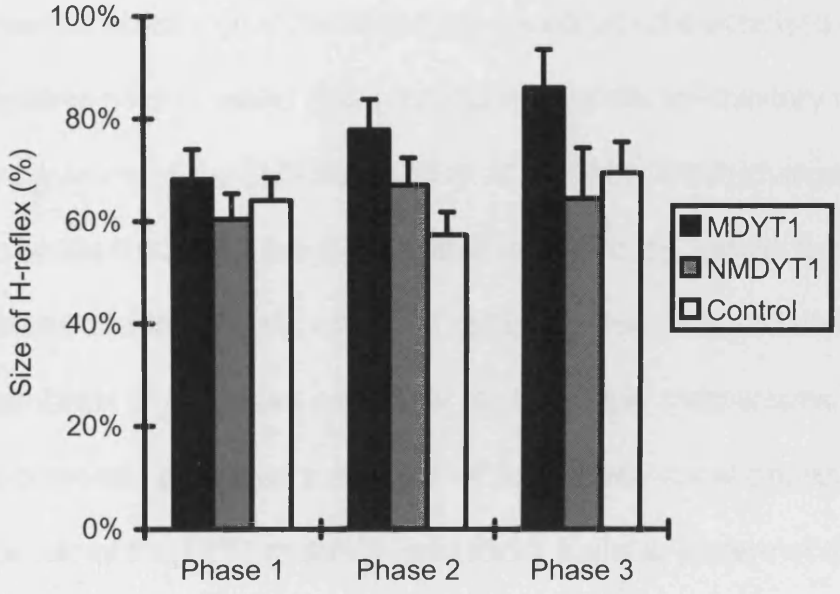
Reciprocal Inhibition (RI)

RI was assessed in 8 MDYT1, 6 NMDYT1 and 13 control subjects. The complete time course of RI at all ISI is shown in figure 5.4a, with grouped data in figure 5.4b. Repeated measures ANOVA was performed with group (MDYT1, NMDYT1 and controls) and ISI as main factors. A significant interaction between group and ISI was found [$F(2,20)=4$, $p<0.05$]. Post hoc analysis on grouped data showed no significant differences between the three groups in the first phase of RI [$F(2,24)=0.441$, ns]. However, a significant difference was found between MDYT1 and controls in the second phase [$F(1,15)=6$, $p<0.05$] and in the third phase [$F=(1,15)=4.6$, $p<0.05$]. NMDYT1 subjects were not significantly different from controls in any of the three phases of RI.

Figure 5.4a shows the H reflex size as a percentage of the unconditioned size at all interstimulus intervals. Figure 5.4b shows mean data for the H reflex size as a percentage of the unconditioned size at each of the three phases of reciprocal inhibition. Error bars indicate standard error of the mean.

A





Discussion

These experiments demonstrated for the first time that electrophysiological abnormalities of cortical excitability exist in both manifesting and non-manifesting carriers of the DYT1 gene. Manifesting and non-manifesting carriers had reduced ICI and shorter cortical silent periods, but the second and third phases of RI were only abnormal in manifesting gene carriers. We conclude that the DYT1 mutation produces subclinical physiological deficits in non-manifesting carriers, which are not as widespread as those seen in manifesting patients. This would be consistent with the hypothesis that additional genetic/environmental insults are necessary to produce clinical dystonia in gene carriers.

Changes in manifesting carriers of the DYT1 mutation.

Previous physiological studies of non-genetically characterised individuals with dystonia have revealed a variety of abnormalities in inhibitory mechanisms at many levels of the CNS(Berardelli et al., 1998b). These changes are thought to be the result of a functional disturbance in basal ganglia function that causes altered thalamic control of cortical motor areas and abnormal regulation of brainstem and spinal cord inhibitory mechanisms. The present experiments examined a selection of cortical and spinal circuits in manifesting carriers of the DYT1 mutation, and found a similar pattern of abnormalities. The reduced ICI is likely to reflect a decrease in the excitability of intrinsic,

probably GABAa, circuits in the motor cortex (Ziemann et al., 1996c) whilst the shorter SP is likely to be due to changes in a different cortical inhibitory circuit that may involve GABAb mechanisms(Ziemann et al., 1996c). Spinal reciprocal inhibition depends in its first part on disynaptic postsynaptic inhibition whereas presynaptic inhibition of Ia terminal is important in its second part. The nature of the third phase of inhibition is unresolved. The present data showing a normal first phase of inhibition and reduced later phases is compatible with the original description of Nakashima et al in non-genetically characterised dystonia(Nakashima et al., 1989).

A criticism of our data in MDYT1 subjects is that some of them were taking medication at the time of the study. Of the ten MDYT1 subjects, five were receiving medication at the time of the study. Two were receiving benzhexol, one clonazepam and benzhexol, one diazepam and one levodopa. It is likely that, if such medication has any effect at all on the parameters measured in our experiments, it would have the effect of *reducing* cortical excitability, not causing the excessive cortical excitability revealed in our experiments. Our results in these medicated subjects did not differ systematically from those not taking medication, and our results overall fit in with established patterns of electrophysiological abnormality found in non-medicated patients with primary dystonia.

Changes in non-manifesting carriers of the DYT1 mutation.

Clinically, movement control in the non-manifesting carriers of the mutation was indistinguishable from that of the normal controls. Despite this, electrophysiological tests revealed subclinical abnormalities: Two GABAergic circuits in the motor cortex were hypoexitable to the same extent as in manifesting individuals, as measured by ICI and SP. Spinal reciprocal inhibition appeared normal.

Previously, it has not been clear why NMDYT1 gene carriers do not manifest dystonia. One potential hypothesis is that the DYT1 gene has no physiological consequences in NMDYT1 individuals, perhaps through inactivation of the gene. Our results would indicate that this is not the case. Clinically non-manifesting carriers of the DYT1 gene had clear electrophysiological abnormalities. In this respect, our data confirm those of Eidelberg et al (Eidelberg et al., 1998) who used PET to reveal subclinical metabolic abnormalities in the brains of non-manifesting individuals. However, our results also show that the abnormalities in NMDYT1 individuals are not as widespread as in MDYT1 patients.

It is interesting that the main abnormalities in NMDYT1 subjects lay in two cortical pathways known to be influenced by basal ganglia input: ICI and SP. This may indicate that the primary defect caused by the DYT1 gene is in basal ganglia function, and that this then leads to secondary changes in connected

structures. Whatever the mechanism, the lack of clinical symptoms in NMDYT1 individuals suggests that there are other factors, perhaps not even tested in these experiments, which determine the expression of clinical dystonia. These factors could be at the level of the sensory system, which has been implicated in the genesis of dystonia, or possibly in the direct connections between the basal ganglia and the brainstem. Regardless of the nature of the additional abnormalities necessary for dystonia to develop, we suggest that genetic and/or environmental modifying factors are likely to play a part in determining the clinical phenotype. There has certainly been considerable debate about the role of environmental factors (particularly trauma) in triggering symptoms in primary dystonia. A recent report of monozygotic twins with familial adult-onset craniocervical dystonia suggested that trauma might have played a role in the greater severity of dystonia in one of the twins (Albanese et al., 2000). Epidemiological studies of patients with blepharospasm have implicated facial trauma as a risk factor for the development of the condition(Defazio et al., 1999). However, little is known about the role of such factors in the onset of dystonia in DYT1 gene carriers. A case-control study (published in abstract form only), implicated measles infection and high fever in early childhood as possible predisposing factors to the development of dystonia in DYT1 gene carriers (Sanders-Pullman et al, 2000). Interestingly, torsin A, the protein product of the DYT1 gene, bears significant homology to heat shock proteins (Breakefield et al., 2001).

The idea that electrophysiological abnormalities may exist without clinical signs of dystonia is not new. Subclinical abnormalities in the unaffected body parts of those with non-genetically characterised primary dystonia have been observed in previous electrophysiological studies. Examples of these abnormalities include abnormal reciprocal inhibition in the forearms of those with cervical dystonia(Deuschl et al., 1992), abnormal intracortical excitability in the hand motor area in those with blepharospasm (Sommer et al., 2002a) or in the unaffected arm of patients with writer's cramp (Ridding et al., 1995), and abnormal temporal discrimination of sensory inputs in the unaffected hand of those with writer's cramp (Fiorio et al., 2003). The implication is that additional abnormalities must occur to prompt appearance of symptoms. In such cases, additional reorganisation of central pathways produced by overuse or injury may be one trigger for dystonia. Thus in these dystonic conditions, as we suspect in DYT1 gene carriers, there also is an interplay between intrinsic and environmental modifying factors that modulates the clinical expression of underlying electrophysiological abnormalities.

In conclusion, we have shown that non-manifesting carriers of the DYT1 gene, although they are clinically unaffected by dystonia, demonstrate some, but not all of the electrophysiological abnormalities found in DYT1 gene carriers with dystonia. This has two implications: first that the electrophysiological changes previously found in those with other forms of dystonia are not merely an artefact of dystonic movements themselves, as they can occur independently of clinical dystonia. Second, it implies that

additional abnormalities are needed to cause clinical dystonia, perhaps in sensorimotor integration or basal ganglia-brainstem outflow. Our findings underline the importance of looking outside cortical motor abnormalities in dystonia to other aspects of the motor system for the clues to the genesis of dystonia in DYT1 gene carriers, and those with other forms of primary dystonia. In addition, it is also important to identify potential environmental and genetic modifying factors that could influence penetrance of the DYT1 phenotype. If these could be identified, it is feasible that DYT1 gene carriers could be protected from, or at least counselled about, such factors. From a wider point of view such factors might give significant insights into the pathogenesis of primary dystonias, and have the potential to provide novel treatment strategies to correct these pathophysiological abnormalities.

Chapter 6

An assessment of the effect of 1Hz rTMS on reciprocal inhibition in DYT1 mutation carriers and normal subjects.

Introduction

This chapter presents details of an experiment designed as an initial exploration of the hypothesis that differential motor system plasticity might underlie the development of dystonia in DYT1 mutation carriers. As discussed above, there is a body of clinical, experimental and theoretical evidence that people with dystonia may be oversensitive to naturally occurring and experimental forces that produce synaptic plasticity. In this current study we set out to assess the ability of an experimental plastic force (1Hz rTMS) delivered over the premotor area to alter a measure of spinal motor inhibitory function: reciprocal inhibition (RI).

We chose 1Hz rTMS as our plastic force in this experiment. This type of low-frequency stimulation has been the most frequently used paradigm in human and animal studies to produce a long-term depressive (LTD) effect on the conditioned tissue. Previous studies in the human motor system have found this effect to be most marked when stimulation is given over the pre-motor, rather than the motor cortex. We hypothesised that an increase in RI might be seen in following rTMS. We expected this effect to be most marked in the

third phase of RI. Even though the exact nature of the third phase of RI is not known, its latency might suggest that it is a spino-bulbo-spinal or even a spino-cortico-spinal loop. As such it might be most susceptible to the effects of rTMS. We hypothesised that manifesting DYT1 carriers (MDYT1) might show the largest effect on RI from rTMS, with perhaps a lesser effect on non-manifesting DYT1 carriers (NMDYT1), and a lesser effect still on normal subjects.

Results

All subjects completed the experiments and none reported any lasting side effects. Paired t tests showed that there was no change in size of the unconditioned H reflex before and after rTMS in control subjects (mean size before rTMS = 1.87mV; mean size after rTMS = 1.61mV; t=1.5, p=0.17) or DYT1 subjects (mean size before rTMS = 0.86; mean size after rTMS = 0.80; t=0.8, p=0.46).

Data for control, NMDYT1 and MDYT1 subjects were grouped for analysis into the three phases of reciprocal inhibition (phase 1:0ms, phase 2:10-20ms and phase 3:75-500ms).

Figure 6.1 shows reciprocal inhibition for control subjects before and after rTMS, figure 6.2 shows data before and after rTMS for MDYT1 subjects, and figure 6.3 shows data before and after rTMS for NMDYT1 subjects.

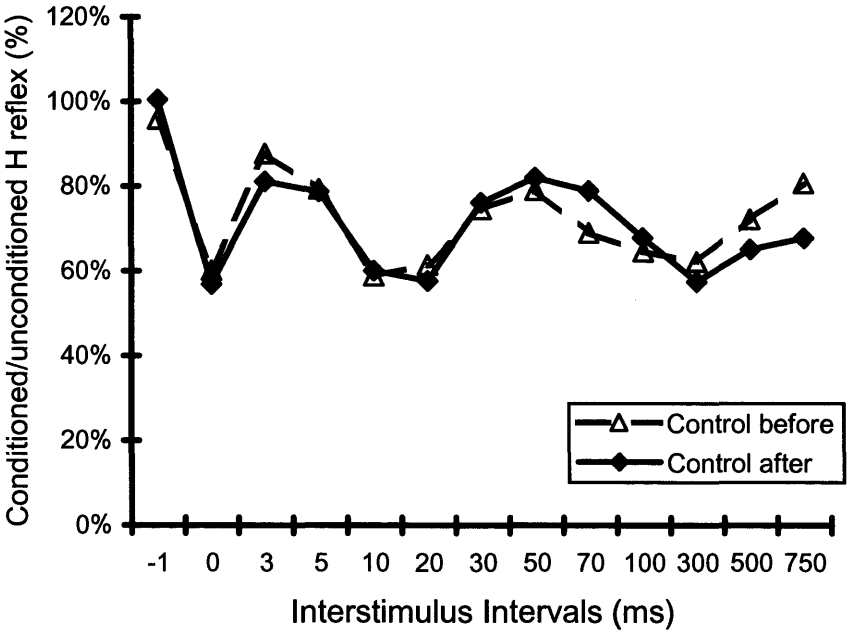


Figure 6.1: Reciprocal inhibition for control subjects before and after rTMS

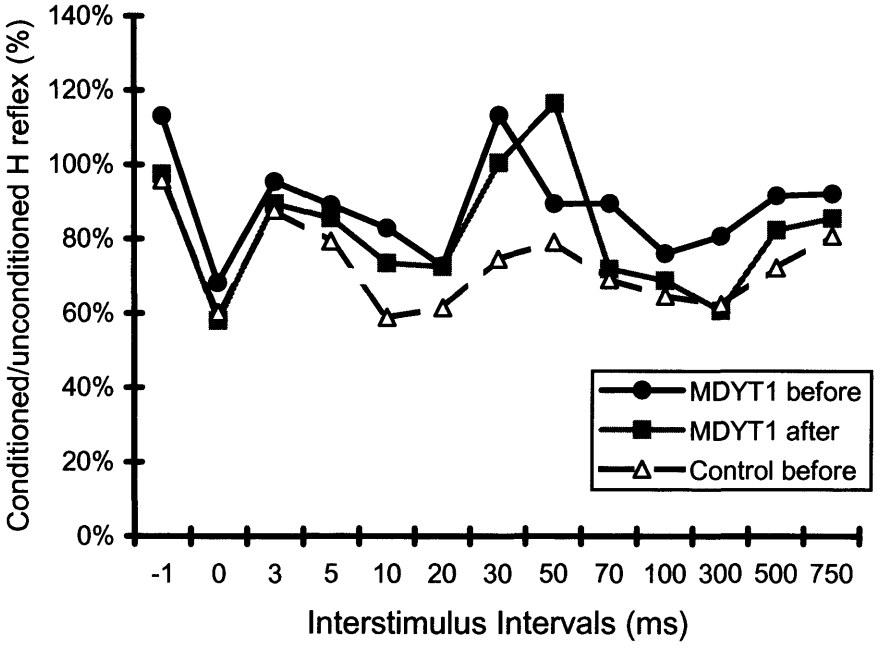


Figure 6.2: Reciprocal inhibition for MDYT1 subjects before and after rTMS, with reciprocal inhibition before rTMS also shown for control subjects.

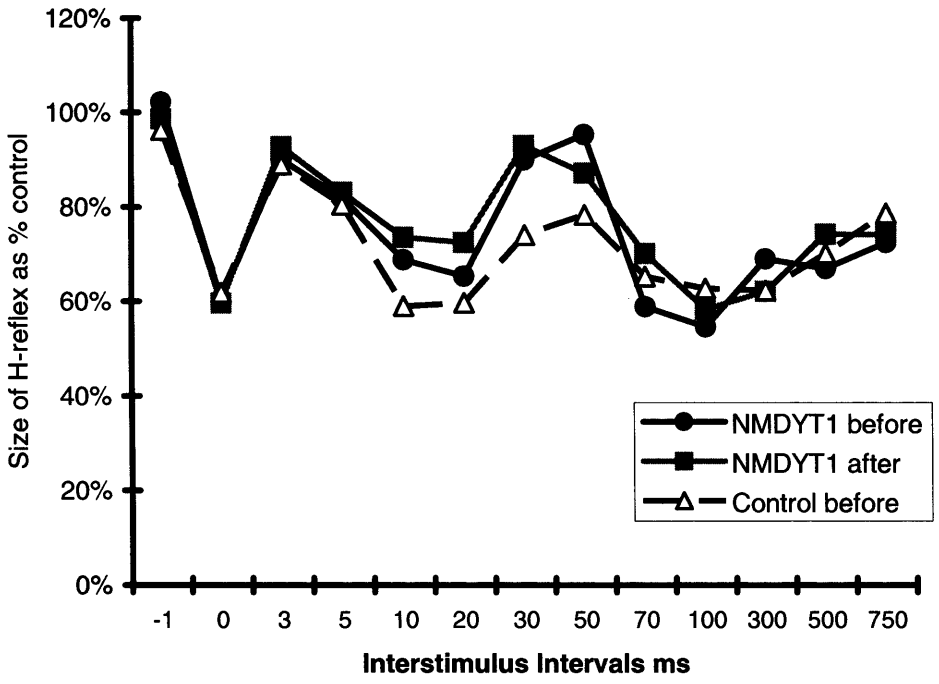


Figure 6.3: Reciprocal inhibition for NMDYT1 subjects before and after rTMS, with reciprocal inhibition before rTMS also shown for control subjects.

Since the mechanisms of the 3 phases of reciprocal inhibition are thought to be different (the first phase is postsynaptic, the second phase presynaptic, and the third phase uncertain at the present time), a two way ANOVA was conducted separately on the data from each phase with TIME (before and after rTMS) and GROUP (controls/MDYT1/NMDYT1) as main factors. In the first phase there was a significant main effect of TIME ($f(1,22)=4.73$, $p<0.05$), but no effect of GROUP and no GROUP x TIME interaction. This was because there was a small but significant increase in the amount of inhibition during this phase after rTMS in MDYT1 subjects (paired t-test, $p<0.05$). In the second phase there was a significant main effect of GROUP ($f(1,22)=5.96$,

$p<0.05$), due to reduced inhibition in MDYT1 subjects. However there was no main effect of TIME and no TIME x GROUP interaction, indicating that rTMS had no effect on this phase of RI in any group. Finally, in the third phase of inhibition there was a significant main effect of TIME ($f(1, 22)=10.54$, $p<0.005$) and a GROUP x TIME interaction ($f(1,22)=11.53$, $p<0.005$). Post hoc t tests (paired t test: $p<0.005$) indicated that this was due to the fact that rTMS increased the amount of RI in MDYT1 subjects but had no effect in controls or NMDYT1 subjects.

Discussion

We have demonstrated that reciprocal inhibition can be modified by 20 minutes of 1-Hz rTMS given over the pre-motor area in subjects with dystonia due to the DYT1 mutation. In these subjects a significant normalisation of the third phase of reciprocal inhibition occurred after rTMS, so that MDYT1 subjects were no longer significantly different in this phase compared to control subjects. The lack of change in the unconditioned H reflex following rTMS is compatible with the idea that there was no direct effect of the rTMS on excitatory spinal motoneurones. NMDYT1 and control subjects had normal reciprocal inhibtion pre rTMS, and did not show any change in inhibition following conditioning.

The first phase of reciprocal inhibition is due to activity in a disynaptic inhibitory pathway (Day et al., 1984) that was once thought to be analogous

to the disynaptic Ia reciprocal inhibitory pathway described in the cat hindlimb (Hultborn, 1976). However, since it does not receive recurrent inhibition from forearm motoneurones, it has been suggested that evolution may have modified the connectivity of reciprocal inhibition to complement the increased circumduction movements that are possible at the human wrist (Aymard et al., 1995). The second phase of inhibition is thought to be due to presynaptic inhibition of the terminals of Ia afferents responsible for the H-reflex (Berardelli et al., 1987). The origin of the third phase is less clear. It has been proposed that it is due to continued presynaptic inhibition, and that the division between second and third phases is caused by superimposition of a short period of facilitation at around 50 ms (Berardelli et al., 1987). An alternative hypothesis is that, due to its long latency, it may involve long loop inhibitory connections from radial nerve to brainstem (spino-bulbo-spinal connections) or even cerebral cortex (transcortical connections) and thence back to the H-reflex pathway in the spinal cord.

Like many other spinal pathways, it is hypothesised that reciprocal inhibition in the forearm is influenced by descending inputs from supraspinal centres that control the excitability of the systems at rest and during movement (Day et al., 1984). Since there is no obvious pathology of the spinal cord in dystonia, reduced inhibition is thought to be due to changes in the level of tonic input from these supraspinal centres, but precisely which centres are affected in dystonia is unknown.

Our data provide two pieces of information regarding these various hypotheses. First, rTMS in dystonic subjects had a differential effect on the second and third phases of reciprocal inhibition, supporting the hypothesis that they are indeed distinct phases with different underlying mechanisms. Second, our data confirm that the pathways that underlie reciprocal inhibition are influenced by changes in the activity of descending inputs. One could speculate that since the premotor cortex has extensive direct and indirect connections to the spinal cord, it may be able to influence the pathways underlying both the first and the third phases of reciprocal inhibition. Alternatively, if the third phase of RI is due to activity in a long-loop spinal-brainstem-spinal pathway then the effect on this phase may be due to changes in activity of premotor-brainstem connections. Via either of these mechanisms, an abnormality in premotor cortex activity may then contribute to the reduced third phase of inhibition in individuals with dystonia, and transient improvement after a period of rTMS may reflect a normalisation of this influence.

We chose to assess the motor hotspot in relationship to the FDI muscle rather than to muscles in the forearm that were involved specifically in the reciprocal inhibition pathway. We hypothesised that as the cortical motor representation of the FDI was so close to such muscles and given the broad field of stimulation provided by a standard TMS coil, that the FDI was a reasonable approximation to the forearm motor hotspot. It is, however, recognised that motor maps in dystonia may be distorted (Byrnes et al., 2005), and this may

therefore be a source of systematic difference between controls and DYT1 subjects. However, the fact remains that DYT1 subjects showed a change in RI with rTMS which presumably must reflect the influence of such stimulation on inhibitory drive to the circuits underlying RI in the forearm.

Our data confirm physiologically Siebner et al's hypothesis (Siebner et al., 2003) that the effects of rTMS at a distance from the site of stimulation may differ in patients and healthy subjects. Thus, stimulation of premotor cortex caused changes in reciprocal inhibitory pathways that were evident in patients but not controls. Although the mechanism behind this differential effect is unclear, it does appear that individuals with abnormal cortical excitability due to disease are more sensitive to the effects of rTMS.

With respect to NMDYT1 subjects, these data provide some limited evidence that the response to a "plastic force" such as rTMS may differ between manifesting and non-manifesting DYT1 subjects. Both groups are known to have abnormal cortical inhibitory function (as assessed by SICI and SP), but at baseline reciprocal inhibition is only abnormal in MDYT1 subjects. Following conditioning there was a clear change in reciprocal inhibition in MDYT1 subjects, but no such change was seen in NMDYT1 subjects. This may be due to differential effects of rTMS in manifesting and non-manifesting subjects (with manifesting subjects being more "sensitive" to a plastic force than non-manifesters). However, due to a floor effect, it may be that it is simply not possible to alter the normal reciprocal inhibition seen in NMDYT1 subjects.

This study therefore provides some preliminary evidence that there may be a differential sensitivity to the effects of rTMS in manifesting and non-manifesting DYT1 carriers.

Chapter 7

A comparison of motor system plasticity in manifesting and non-manifesting DYT1 carriers, subjects with adult-onset dystonia and controls.

Introduction

The last chapter presented details of an experiment using rTMS as an experimental "plastic force" in DYT1 mutation carriers. In this experiment we used a test of spinal motor inhibitory function (spinal reciprocal inhibition: RI) to assess the response of subjects to rTMS. We found a significant normalisation of RI in MDYT1 subjects, but no change in NMDYT1 or controls.

These results could be interpreted as suggesting that response to rTMS is excessive in MDYT1 subjects compared to NMDYT1 and normal controls as they are the only subject group to demonstrate a change in RI following conditioning. One could conclude that excessive synaptic plasticity is therefore a primary abnormality in those who manifest dystonia. However, there are a number of confounding factors that might affect this conclusion. Firstly, MDYT1 subjects were to only group to have abnormal RI at baseline. One could argue that because RI is abnormal, it might be easier to change it with an intervention such as rTMS, compared with the situation where it is normal as in NMDYT1 and controls. Secondly, it is possible that an excessive

response to a plastic force such as rTMS occurs as a consequence of dystonic movements being present for a number of years; i.e. the abnormal response to rTMS is a secondary phenomenon, and not indicative of a primary driving force behind the development of dystonia.

We therefore wished to assess synaptic plasticity in the motor system in a different way, not so open to these confounding factors. We therefore chose to assess change in MEP size following rTMS in our subjects. Motor thresholds are not different in those with dystonia compared to normal subjects. As described in chapter 3 we found abnormalities in cortical inhibition that were similar in severity between MDYT1 and NMDYT1 subjects.

Results

No side effects were observed or reported by any subject following rTMS. All subjects completed the experiment.

As expected, we found that normal subjects had a significant decrease in MEP size following rTMS which lasted for approximately 20 minutes (fig. 1). In contrast, manifesting DYT1 subjects had a significantly prolonged response to rTMS, with MEP size still maximally suppressed at the end of assessment (fig. 1).

Figure 7.1

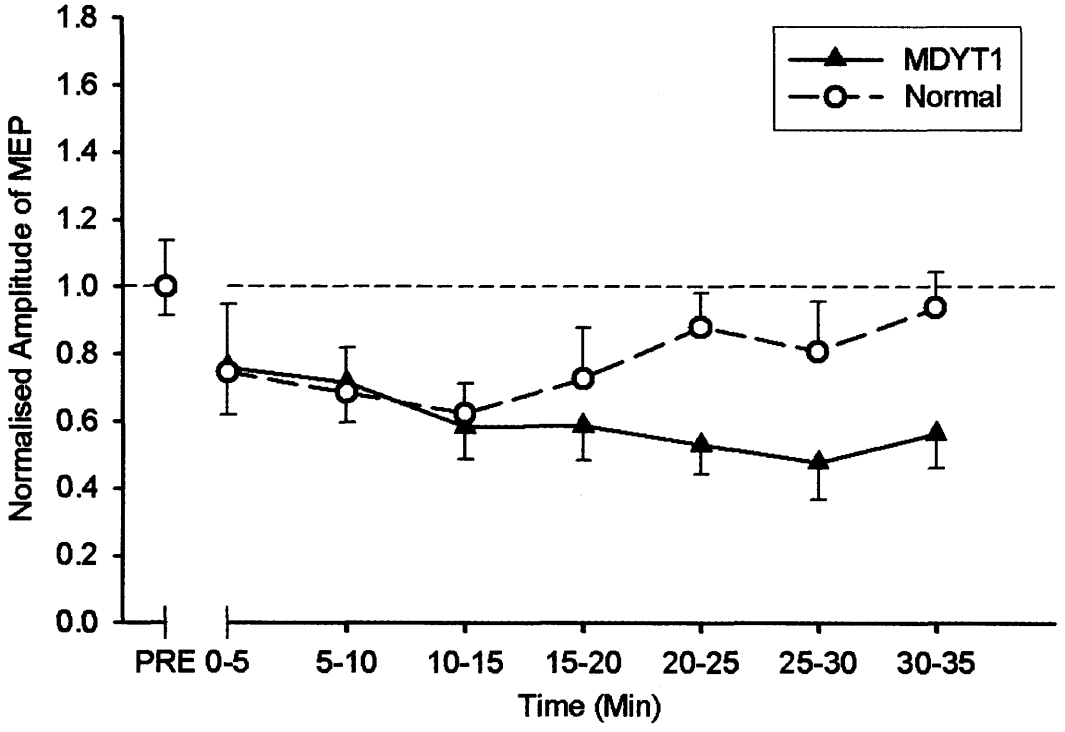


Figure 7.1:Normalised MEP size at baseline and following rTMS in normal subjects and manifesting DYT1 mutation carriers (MDYT1). 2 way ANOVA revealed a significant TIME x GROUP interaction ($F(7,84)=2.99$, $p<0.01$). Post hoc analysis revealed this to be due to a significantly prolonged suppression of MEP size after 20 minutes in MDYT1 subjects compared to controls ($p<0.01$). Error bars indicate standard error.

A similar pattern was observed in subjects with sporadic, adult-onset dystonia, who also had a significantly prolonged suppression of MEP size following rTMS (fig. 7.2).

Figure 7.2

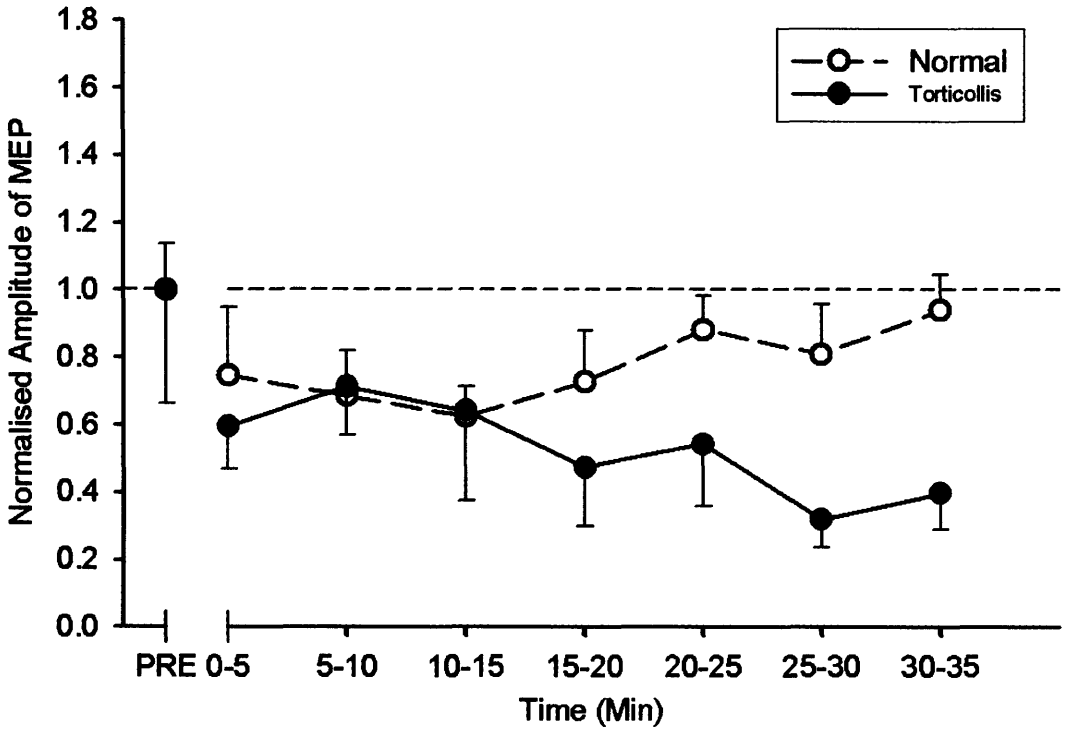


Figure 7.2: Normalised MEP size at baseline and following rTMS in normal subjects and subjects with adult-onset neck dystonia (torticollis). 2 way ANOVA revealed a significant TIME x GROUP interaction ($F(7,77)=3.84$, $p<0.001$). Post hoc analysis revealed this to be due to a significantly prolonged suppression of MEP size after 20 minutes in torticollis subjects compared to controls ($p<0.01$). Error bars indicate standard error.

Non-manifesting DYT1 subjects were also abnormal in the their response to rTMS, but in the *opposite* direction, showing no significant change in MEP size at any time point after rTMS, significantly different in this respect from both normal and dystonia subjects (fig. 7.3).

Figure 7.3

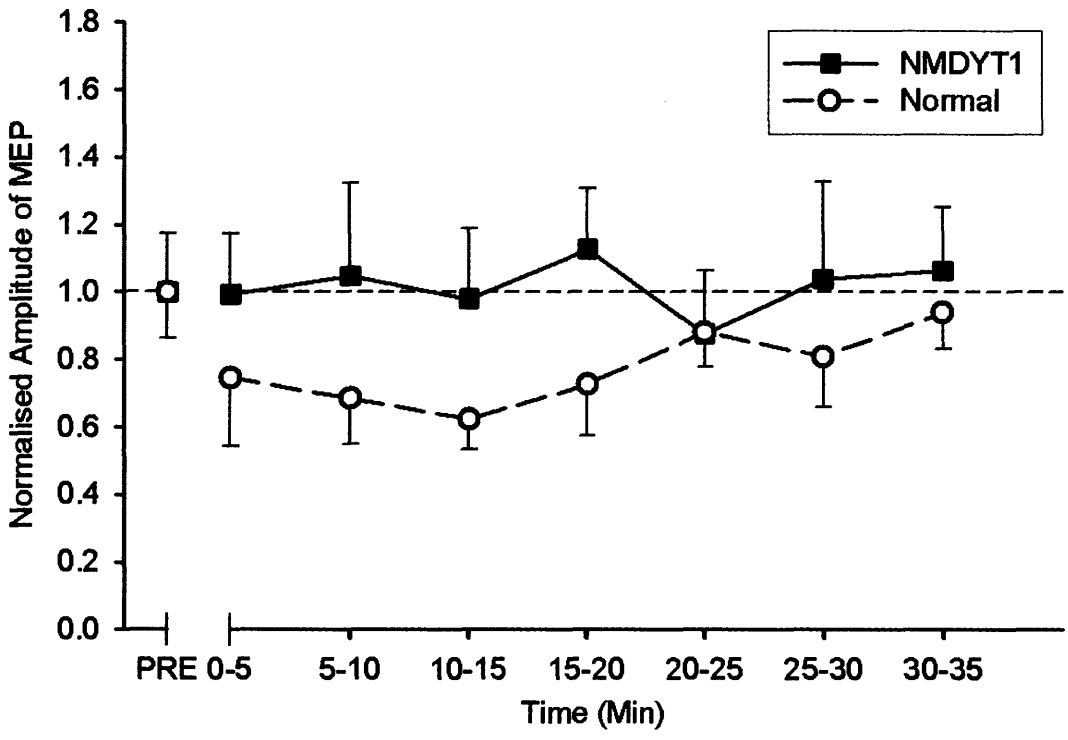


Figure 7.3: Normalised MEP size before and after rTMS in normal subjects and non-manifesting DYT1 mutation carriers. 2 way ANOVA revealed a significant TIME x GROUP interaction ($F(7,77)=2.22$, $p<0.05$). Post hoc analysis revealed this to be due to significantly reduced MEP suppression in NMDYT1 subjects compared to controls. Error bars indicate standard error of the mean.

Discussion

We have demonstrated that an abnormal response to an experimental plastic force occurs in individuals who are genetically susceptible to dystonia, and that the direction of the abnormal response is determined by the presence or absence of clinical symptoms. Thus in DYT1 mutation carriers with dystonia

(and also in sporadic adult-onset dystonia) the response to induction of plastic change in the motor cortex is excessive. One could argue that this abnormal response occurs secondary to changes in the motor system caused by long-standing dystonia. However, the presence of a *sub*-normal response to the attempted induction of plastic change in the motor cortex in non-manifesting DYT1 subjects suggests a primary role for brain plasticity in the development of clinical dystonia. The subnormal responsiveness of the system underlying plastic change in the brain observed in non-manifesting DYT1 carriers may have implications for other brain functions that require plastic changes to occur, and in this regard it is interesting that a deficit in the ability to perform a motor sequence learning task has been reported in non-manifesting DYT1 mutation carriers (Ghilardi et al., 2003).

It would be of considerable importance to determine which aspect(s) of the complex system that regulates brain plasticity determines the differential response to rTMS observed in DYT1 mutation carriers. In experimental models of plasticity, modulation of GABA (Chen et al., 1994) and dopamine (Kusuki et al., 1997) can alter the direction of plastic change (LTP vs. LTD) in response to a particular pattern of stimulation. GABA would appear to be an unlikely candidate to explain the difference in motor cortex plasticity in DYT1 mutation carriers, as electrophysiological tests designed to probe the function of GABA in the motor system are similarly impaired in manifesting and non-manifesting carriers (see chapter 5). Dopamine may prove to be a more promising contender. Although there is no loss of dopaminergic cells in DYT1

dystonia, torsin A is known to be maximally expressed in dopaminergic neurons (Augood et al., 1999; Konakova et al., 2001) and has been linked to the function of VMAT2, a protein controlling the quantal release of dopamine at the synapse (Misbahuddin et al., 2005). Dopamine turnover in the brains of those with manifesting DYT1 dystonia may be increased (Augood et al., 2002), and recently, abnormalities in post synaptic dopamine receptors have been found in DYT1 carriers (Asanuma et al., 2005). Increased concentrations of dopamine promote LTP, and one might speculate that in manifesting DYT1 carriers, abnormal (excessive) dopamine release in response to natural stimuli that can promote plastic changes in the motor cortex, produce an excessive response. This could lead to excessive excitability of motor pathways, and therefore loss of the normal pattern of centre-surround inhibition that characterises normal motor system operation. In non-manifesting DYT1 carriers, one would have to hypothesise a compensatory mechanism that mitigates this deficit, perhaps mediated by another genetic factor. Future studies to identify the mechanism of differential plasticity in DYT1 carriers could determine potential therapeutic targets capable of being exploited to modulate the emergence of clinical dystonia in susceptible individuals.

CHAPTER 8

Conclusions

Individuals who carry the DYT1 gene mutation have a 30-40% chance of developing dystonia, and do so in an age-dependent fashion (Bressman et al., 2000). The unique model provided by this natural occurrence has allowed us to identify those aspects of motor system dysfunction are may be fundamental to the production of clinical dystonia, and which aspects may be protective against the development of symptoms in genetically susceptible individuals.

We have shown that abnormalities in cortical motor inhibition can be present to the same severity in manifesting and non-manifesting carriers of the DYT1 mutation, demonstrating that they are not sufficient on their own to cause dystonia to manifest. We developed a new hypothesis regarding the manifestation of symptoms in DYT1 mutation carriers based on abnormalities in synaptic plasticity in the motor system. We demonstrated excessive synaptic plasticity in manifesting mutation carriers, but sub-normal plasticity in non-manifesting carriers. These data place abnormalities in the sensitivity of the "synaptic plasticity system" at the heart of a model to explain the pathogenesis of DYT1 dystonia, and by inference, primary dystonia in general.

The next key step in the understanding of dystonia pathophysiology would be to identify which part(s) of the complex mechanism controlling the sensitivity of synaptic plasticity drives the different response of manifesting and non-manifesting carriers to rTMS. This aspect might well provide a pathway towards the ability to manipulate, for therapeutic gain, synaptic plasticity in manifesting carriers, perhaps even at a pre-symptomatic stage. It might also be of use to those with commoner forms of primary dystonia in whom a related mechanism may be responsible.

It seems likely that any therapeutic intervention that does not take account of abnormal synaptic plasticity in people with dystonia is likely to fail in the long term. Thus, although benefit has been reported from re-training methods in dystonia patients (Cabrera-Lopez et al., 2003; Candia et al., 1999; Candia et al., 2002; Zeuner et al., 2002), it seems likely that the underlying tendency towards excessive plastic changes will undo any benefit from such retraining techniques. This seems particularly likely in those who are re-exposed to the same stimulus that originally triggered the dystonia such as professional musicians. Such people might benefit from re-training coupled with a method to prevent excessive plastic changes happening once retraining has finished.

rTMS is one method of experimentally inducing plastic change. Traditional methods of rTMS have some disadvantages as a therapeutic intervention: they are slow to produce changes, changes are subject to inter and intra individual variation (Sommer et al., 2002b), and technical problems (e.g. high

resting threshold) can interfere with conditioning. The technique of theta burst stimulation (Appendix 1) offers a faster and more consistent method of inducing plastic change using rTMS, and may therefore be more suitable as a therapeutic tool.

It is possible that rTMS, or other methods of experimentally inducing plastic change such as IPAS can be combined with re-training to produce long lasting "remapping" of distorted motor maps in dystonia, and therefore lasting clinical benefit.

This study has shown that synaptic plasticity can be both a protective and a damaging force. The task ahead is to understand how this system can be controlled, and its great powers harnessed for the benefit of those with dystonia.

APPENDIX 1: Details of a novel paradigm of rTMS in humans: Theta Burst Stimulation.

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Introduction

In animal experiments it has long been possible to probe and manipulate the efficacy of synaptic transmission by repetitive electrical stimulation of central nervous pathways. This leads to the well-studied phenomena of long term potentiation (LTP) and depression (LTD) of synaptic connections. Repetitive transcranial magnetic stimulation (rTMS), which is a non-invasive method of stimulating the brain of conscious human subjects through the intact scalp, has obvious potential for mimicking the effects that have been observed in animal models. Yet despite the striking effects on synaptic transmission that have been achieved in animals, translation to the human brain using rTMS has been relatively disappointing.

Investigations have been carried out on three levels: physiological, behavioural and clinical. All are designed to detect changes in function that outlast the application of particular patterns of rTMS to selected areas of cortex. The majority of physiological studies have employed the motor cortex since it is possible to use the size of the electromyographic (EMG) response to

a single TMS pulse as an objective measure of cortical excitability. Here, results are often weak, highly variable from one individual to another (Maeda et al., 2000a), and rarely last longer than half an hour. Behaviourally, the experiments on the motor system produce no obvious effects on basic motor parameters such as strength or speed of contraction (Muellbacher et al., 2000). However, small changes can be seen in more complex paradigms. Similarly, rTMS over other cortical areas can induce subtle changes in cognitive functions (Evers et al., 2001; Hadland et al., 2001; Sparing et al., 2001), but again these are relatively modest. Clinically, rTMS has been used to try to treat a variety of neurological and psychiatric conditions from Parkinson's disease to obsessive compulsive disorder. The largest number of trials has been for depression, but again, the results have been equivocal (Hausmann et al., 2004; Martin et al., 2003).

There are several possible reasons for the previous disappointing results of rTMS: first, even in animal experiments, LTP/LTD is difficult to demonstrate in the cortex of awake and freely moving animals without the use of extended or repeated sessions of stimulation (Froc et al., 2000; Trepel and Racine, 1998). Second, concerns over safety have limited many humans studies to relatively low frequencies of stimulation (usually <10 Hz) (Wassermann, 1998) whereas animal studies often use much higher frequencies such as the "theta burst" paradigm (3-5 pulses at 100 Hz repeated at 5 Hz) (Hess et al., 1996; Huemmeke et al., 2002; Larson and Lynch, 1986; Vickery et al., 1997). Third,

TMS in humans is relatively non-focal, and therefore cannot be used to target spatially specific neural connections. In most instances, this means that rTMS will activate a mixture of systems that potentially could have interacting effects that make the final outcome difficult to predict.

Other stimulation methods have been used to try to induce plastic changes in human cortex, for example paired associative stimulation (PAS) (Ridding and Uy, 2003; Stefan et al., 2000), or transcranial direct current stimulation (Nitsche and Paulus, 2000). PAS can produce controllable change in cortical excitability, but protocols typically require periods of conditioning of around 30 minutes, and peripheral stimulation is given at 2-3 times sensory threshold which may be uncomfortable for some subjects. There is less experience with the use of tDCS, and again conditioning times of over several minutes typically are needed to produce any effect.

A recent pilot study has shown that a single short, low intensity burst of rTMS at 50 Hz is safe and can target specific populations of neurones in the motor cortex (Huang and Rothwell, 2004). In the present experiments we have aimed to produce clear after effects of rTMS in the human motor cortex, by employing repeated application of such bursts in modified "theta burst" paradigms (TBS).

Experimental Procedures

Subjects

Subjects were nine healthy volunteers between the ages of 23 and 52 (mean age: 33.6±7.8 years) who gave their informed consent for the experiments. The project protocol was approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery.

Stimulation and Recording

Subjects were seated and EMGs recorded with a gain of 1000 and 5000 using Ag-AgCl surface electrodes over the right first dorsal interosseous muscle (dominant hand in all subjects). Magnetic stimulation was given over the hand area of the motor cortex using a hand-held figure of eight coil (70 mm standard coil, Magstim Co., Whitland, Dyfed, UK) placed tangentially to the scalp with the handle pointing posteriorly. Single and paired pulses were delivered by Magstim 200 machines, and rTMS was delivered using a Magstim Super Rapid stimulator. The stimulation intensity was defined in relation to the active motor threshold (AMT) for each Magstim machine separately as the minimum single pulse intensity required to produce an MEP of greater than 200μV on more than five out of ten trials from the contralateral FDI while the subject was maintaining a voluntary contraction of about 20% of maximum using visual feedback.

Experiments

The patterns of rTMS all consisted of bursts containing 3 pulses at 50Hz and an intensity of 80% AMT repeated at 200ms intervals (i.e. at 5Hz). In the intermittent theta burst stimulation pattern (iTBS), a 2s train of TBS is repeated every 10s for a total of 190s (600 pulses). In the intermediate theta burst stimulation paradigm (imTBS) a 5s train of TBS is repeated every 15s for a total of 110s (600 pulses). In the continuous theta burst stimulation paradigm (cTBS) a 40s train of uninterrupted TBS is given (600 pulses). (Fig. 1A) An additional comparison was made in some subjects with regular 15Hz stimulation at the same intensity.

Corticospinal excitability was assessed by measuring the peak-to-peak amplitude of MEPs in the contralateral FDI muscle to single pulse TMS in resting subjects. Before TBS 30 pulses were given every 4.5-5.5s. After TBS, batches of MEPs to 12 single pulses were measured at different intervals.

To better understand the mechanism of our different TBS paradigms, we explored the effect of a single train of 10 and 25 bursts given over the motor hand area. MEPs were accessed 4-5 seconds before the train of bursts and at 1 second, 5 seconds, 10 seconds, and 15 seconds after the train in one block of testing. The block was then repeated every 40-45 seconds for 10 repeats. Two separate sessions using either a 10 bursts or a 25 burst train were

assessed in each subject. Five subjects (3 men, 2 women; mean age, 27±5 years) were recruited in this part.

We assessed short interval intracortical inhibition (SICI) and facilitation (ICF) in the motor hand area of seven subjects before and after TBS using the double-pulse method described by Kujirai et al (Kujirai et al., 1993). SICI was evaluated at an interstimulus interval (ISI) of 2ms using a conditioning intensity of 80% AMT, and ICF at an ISI of 10ms with a conditioning intensity of 90% AMT. Two blocks of baseline SICI and ICF were recorded with 10 trials of each condition randomly intermixed with controls. The RMT was increased from 49.0±8.9% to 51.0±9.7% of maximum output of the magnetic stimulator ($t=-3.24$, $p<0.05$) by cTBS, while AMT stayed unchanged ($t=0.55$, ns). We therefore adjusted the intensity of the test stimuli while assessing SICI and ICF after TBS to maintain the amplitude of test MEPs at approximately 1 mV, but left the conditioning intensity unchanged.

We also tested the H-reflex and MEP in the contralateral flexor carpi radialis (FCR) muscle before and after cTBS on seven subjects. One block mixing 12 trials of H-reflex and 12 trials of MEP was recorded prior to conditioning, and another block was recorded at 10 min after cTBS.

In a separate experiment, we assessed reaction time before and after cTBS in nine subjects. Subjects were seated in a comfortable chair with each index finger placed on a button. An electrical stimulus at an intensity of 3 times sensory threshold was delivered randomly to the left or the right hand through Ag/Ag-Cl electrodes attached over the hypothenar eminence. Subjects were instructed that when they felt a stimulus on the right or the left hand, that they were to press the button under the corresponding finger as quickly as possible. In addition, subjects were asked to press the button with a particular force (approximately 2.5 N) with respect to visual feedback given on a screen in front of them.

Two blocks of reaction time testing were performed, with 40 stimuli to each hand given at random intervals, ranging from 1.5 to 2.5 seconds, and in a random pattern. cTBS was then given over the left motor hand area, and the process repeated at 10 and 30 min.

Data analysis

Data were analysed using SPSS for Windows version 11.0. Repeated measures ANOVA was used to compare variables before and after TBS, and paired t-tests were used to compare the effect of TBS on H-reflexes and MEPs recorded from FCR and the effect of a single pulse. Statistics for the data in Fig. 1 comparing the effect of iTBS, imTBS and cTBS were performed on

normalised data, whereas the statistical analysis of each time course separately was performed on absolute values. The comparison of data between MEP and H reflex was performed on log transformed values in order to normalise the distribution of the amplitude data. All figures represent group data. Error bars refer to the standard error of the measurements.

Results and Discussion

In the first experiment three patterns of TBS (Fig. 1A), each consisting of a total of 600 pulses at an intensity of 80% active motor threshold were given on different days to the primary motor cortex of same group of subjects. The basic element of all of these patterns was a burst of 3 stimuli at 50 Hz (i.e. 20ms between each stimulus) which was repeated at intervals of 200ms (i.e. 5 Hz). We refer to these patterns as continuous TBS (cTBS), intermittent TBS (iTBS) and intermediate TBS (imTBS). The excitability of the corticospinal system before and after TBS was measured using single pulse TMS to evoke EMG responses (motor evoked potentials, MEPs) in a small hand muscle. Fig. 1B shows that after cTBS MEPs were suppressed for over 20 min, whereas they were unaffected after imTBS and facilitated after iTBS (ANOVA: significant effect of PATTERN (i.e. iTBS, imTBS, or cTBS) ($f(2,16) = 20.32$, $p < 0.001$) with significant post hoc differences between each pair of TBS patterns). Fig. 1C shows that the duration of the after effects was shorter when fewer TMS pulses were applied in the cTBS pattern. MEPs were suppressed for 60 min after a total of 600 pulses (i.e. 40s cTBS) whereas they

were suppressed for only 20 min after 300 pulses (i.e. 20s cTBS) (ANOVA: significant TIME x DURATION interaction ($f(14,112)=2.24$, $p<0.05$). In a subset of 6 subjects we extended the period of measurement beyond 60 min in order to confirm that the effects of 40s cTBS had returned to baseline after 1 hour (Fig 1D). The one way ANOVA on this data revealed a significant effect to TIME ($f(3,15)=4.36$, $p<0.05$), with post hoc tests showing significant suppression of MEPs at 25 and 45 min but not at 61 and 65 min.

In order to understand which features of TBS patterns are critical to the observed after effects, we compared the results of applying 300 TMS pulses continuously at 15 Hz with the same number of pulses in the cTBS pattern. Although it took 20s to apply each type of conditioning, only the cTBS pattern had any after effect on the responses to TMS (Fig. 1E) (significant interaction between TIME and PATTERN ($f(14,84)=2.55$, $p<0.005$), confirming the importance of the high frequency burst component of TBS for producing long-lasting after effects.

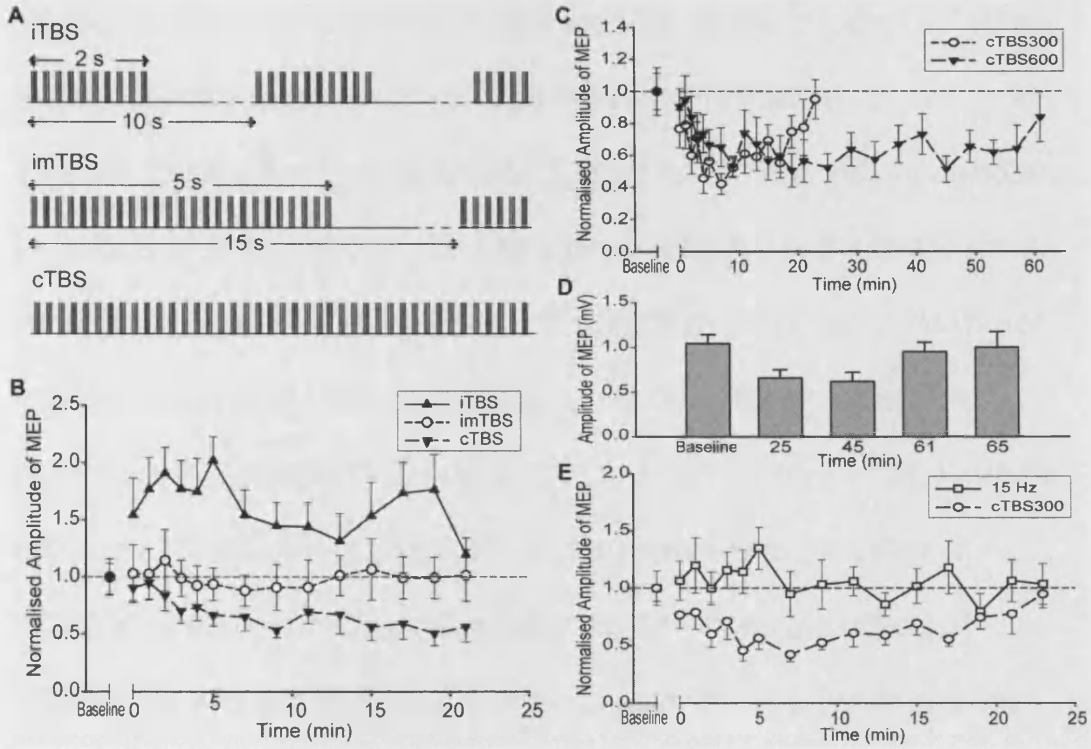

Figure 1 A-E: *Paradigms of TBS and their effects on MEPs. Fig. 1A gives a graphical illustration of the three stimulation paradigms used. Each paradigm uses a theta burst stimulation pattern (TBS) in which 3 pulses of stimulation are given at 50Hz, repeated every 200ms. In the intermittent theta burst stimulation pattern (iTBS), a 2s train of TBS is repeated every 10s for a total of 190s (600 pulses). In the intermediate theta burst stimulation paradigm (imTBS) a 5s train of TBS is repeated every 15s for a total of 110s (600 pulses). In the continuous theta burst stimulation paradigm (cTBS) a 40s train of uninterrupted TBS is given (600 pulses). Fig. 1B shows the time course of changes in MEP amplitude following conditioning with iTBS (▲), cTBS (▼), or imTBS (○). There was a significant effect of pattern of stimulation on change in MEP size following stimulation ($f(2,16)=20.32$, $p<0.001$), with significant post hoc differences between each pattern of stimulation. There was a*

significant facillitation of MEP size following iTBS lasting for about 15 mins, and a significant reduction of MEP size following cTBS lasting for nearly 60 mins. im TBS produced no significant changes in MEP size. Fig. 1C compares the effects of cTBS given for 20s (300 pulses; cTBS300 (○)) with the same paradigm given for 40s (600 pulses; cTBS600(▼)). There was a significant effect of duration of cTBS conditioning on the time course of the effect (significant TIMExDURATION interaction ($f(14,112)=2.24$, $p<0.05$)) with the effect of cTBS300 lasting about 20 minutes compared to the effect of cTBS600 which lasted about 60 minutes. Fig 1 D shows the effects of cTBS600 on a longer time scale in order to confirm the return to baseline levels after 1 hour. Data are from 6 subjects and show suppression at 25 and 45 min but no effect at 61 and 65 min. Fig. 1E compares the effect of continuous 15Hz stimulation for 20s (□) (300 pulses) with cTBS given for 20s (○) (300 pulses). Only the cTBS paradigm had any effect on MEP size following stimulation, and there was a significant interaction between TIME and PATTERN ($f(14,84)=2.55$, $p<0.005$). This graph also shows more clearly than Fig. 1C that the effect of cTBS300 had returned to baseline by 20 min.

A second experiment compared the effect of applying a single train of TBS for either 2s (i.e. the individual component of the iTBS pattern) or 5s (the component of the imTBS pattern). Fig. 2A shows that as expected from the small total number of pulses applied, these short trains produced after effects that lasted only 15s or so. However, a 2s train had a purely facilitatory effect on MEPs (Fig. 2A), whereas MEPs were initially facilitated after a 5s train, but

then suppressed at 10s before returning to baseline at 15s (Fig. 2B). Given that a 20s train of TBS (i.e. the cTBS pattern) is purely suppressive, this suggests that a single train of TBS can lead to a mixture of suppressive and facilitatory effects on MEPs, with facilitation building up faster than suppression, but with suppression being more powerful in the long term.

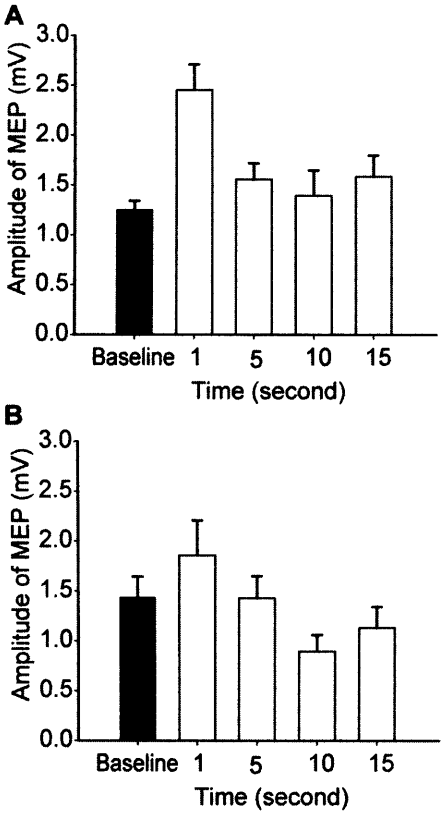

Figure 2A and 2B: *The effect on MEP size of a short burst of TBS. MEP size was measured at baseline and then at 1, 5, 10 and 15s following the end of stimulation. Following a 2s train of TBS (Fig. 2A) there was a significant facilitation of MEP size ($f(4,16)=6.99$, $p<0.005$). In contrast a 5s train of TBS (Fig.2B) produced an initial significant facilitation of MEP size at 1 second*

after the end of stimulation ($p<0.05$) followed by a significant suppression of MEP size at 10s ($p<0.05$).

Given the very low intensity of the individual pulses used in the conditioning trains (80% AMT), it is unlikely that TBS produced any activity in descending corticospinal fibres, and therefore that there were any direct effects of TBS on the excitability of circuits in the spinal cord that could contribute to the MEP changes that were observed. Consistent with this, we found that cTBS with 300 pulses had no effect on H reflexes evoked in forearm flexor muscles whereas MEPs were suppressed (ANOVA on log transformed amplitude data of H-reflex and MEP: significant interaction between TIME and RESPONSE TYPE ($f(1,7)=6.05$, $p<0.05$).

To confirm that TBS has an effect on the excitability of circuits intrinsic to the motor cortex, we measured short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) before and after iTBS and cTBS300 using a paired pulse paradigm. In these experiments, the intensity of the second, test, stimulus was adjusted so that it evoked the same size of baseline MEP before and after TBS. Fig. 3A, B shows that SICI was significantly facilitated following iTBS (ANOVA on the time course: $f(4,24)=5.01$, $p<0.005$) and suppressed after cTBS ($f(5,30)=3.75$, $p<0.01$). In contrast, ICF was

unaffected by iTBS and slightly reduced 10 minutes after cTBS300 ($f(2,12)=7.40$, $p<0.01$) (Fig. 3C, D).

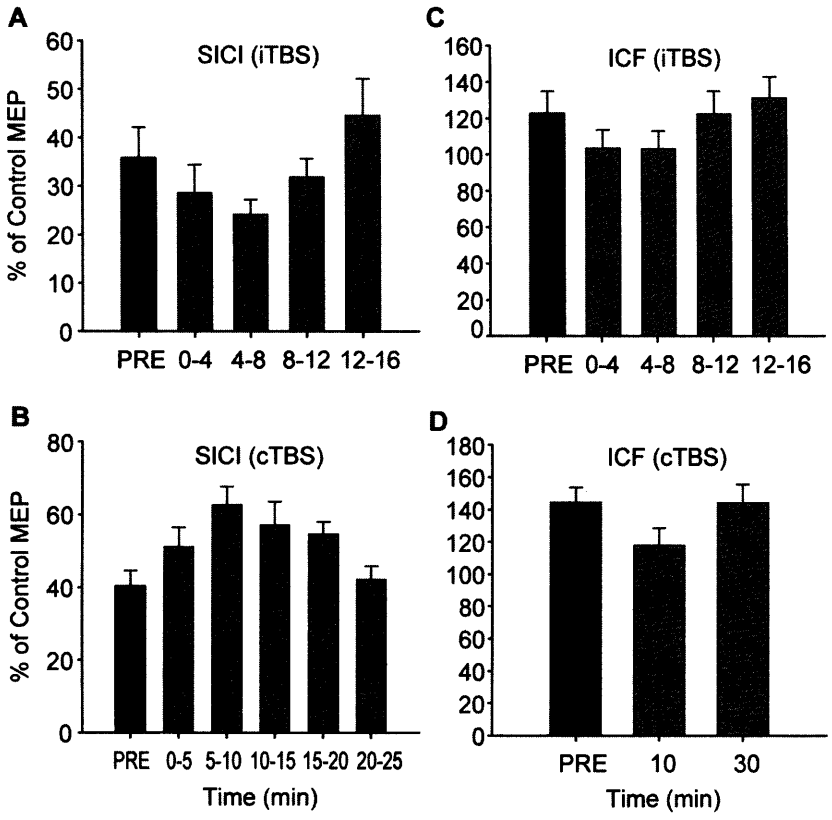

Figure 3 A-D: *The effect of iTBS and cTBS on short intracortical inhibition (SICI) and facilitation (ICF). (A) SICI was significantly increased following iTBS ($f(4,24)=5.01$, $p<0.005$), but (B) was reduced following cTBS ($f(5,30)=3.75$, $p<0.01$). (C) ICF was not significantly altered following iTBS, but (D) was significantly reduced at 10 mins following cTBS ($f(2,12)=7.40$, $p<0.01$).*

Unlike most other methods of conditioning the motor cortex (Chen et al., 1997; Muellbacher et al., 2000), cTBS with 300 pulses in total produced clear

changes in simple reaction times. In this experiment, cTBS300 was applied to the left motor cortex and reaction times measured in the right (conditioned) and left (unconditioned) hands (Fig. 4). A two factor ANOVA revealed a significant interaction between time (before and after cTBS300) and hand ($f(2,16)=4.30$, $p<0.05$.) indicating that cTBS300 had a different effect on the reaction times of the two hands. One factor analyses showed that there was a significant effect of time in both hands (conditioned hand: $f(2,16)=12.77$, $p<0.001$; unconditioned hand: $f(2,16)=7.82$, $p<0.005$) However, in the unconditioned hand this was due to a decrease in reaction times 30 min after cTBS300, whereas in the conditioned hand it was due to an increase in reaction time 10 min after cTBS300. The accuracy of the force with which subjects pressed the button was not changed in either hand following conditioning (conditioned hand: ($f(2,16)=0.18$, ns; unconditioned hand: $f(2,16)=1.14$, ns)

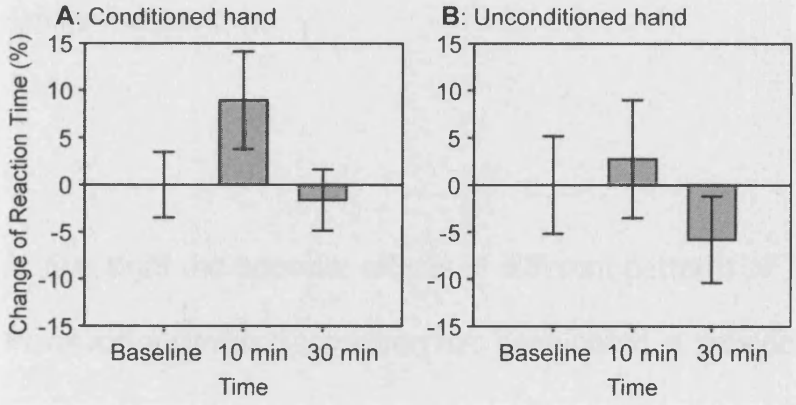

Figure 4A and 4B: *Fig. 4 illustrates the changes in simple reaction time following cTBS. There was a significant lengthening of reaction time in the conditioned hand 10 min after cTBS ($f(2,16)=4.30$, $p<0.05$; Fig.4A), and a*

significant shortening of reaction time in the unconditioned hand 30 min after cTBS ($f(2,16)=7.82$, $p<0.005$; Fig. 4B).

These data confirm that very short periods of low intensity TBS over motor cortex can have powerful effects on physiology and behaviour that outlast the conditioning by up to 1 hour. Since spinal H-reflexes were unaffected whereas two sets of intracortical circuitry tested by SICI (a probably GABAa-ergic pathway (Chen et al., 1998; Hanajima et al., 1998; Reis et al., 2002; Ziemann et al., 1998)) and ICF (pathway unknown) were clearly modulated, it seems likely that TBS was exerting its main effects on the excitability of neurones in the motor cortex. Given that there is now good evidence that other forms of TMS conditioning produce their after effects by changing the effectiveness of synaptic interactions (Lee et al., 2003; Siebner et al., 2003; Siebner et al., 2000), we believe that the present results are compatible with induction of similar mechanisms.

At first sight the opposite effects of different patterns of TBS are surprising. However, a similar dissociation has been noted in previous work on animal preparations: patterns of intermittent TBS similar to our iTBS paradigm are routinely used to facilitate synaptic connections(Capocchi et al., 1992; Hess and Donoghue, 1996; Heynen and Bear, 2001), whereas a small number of studies have used longer trains of TBS-like paradigm to produce suppression

(Heusler et al., 2000; Takita et al., 1999). Our data would be compatible with similar mechanisms in which cTBS might reduce the efficacy of transmission through the synaptic connections that are recruited when evoking an MEP (i.e. the I wave circuits) whereas iTBS would have the opposite effect. Similar arguments can account for the changes in SICI and ICF that we observed. Thus, we suggest that cTBS decreased the effectiveness of synaptic connections that are recruited in circuits involved in both SICI and ICF. This would reduce SICI, resulting in less MEP inhibition probed by SICI, and also reduce MEP facilitation probed with ICF. Conversely, iTBS, which facilitated MEPs might also increase the effectiveness of connections involved in SICI and increase MEP suppression probed by SICI. There was no corresponding facilitation of the SICF circuit in the present data after iTBS. The reason for this is unclear, but it may be related to the fact that more than one circuit contributes to ICF (Hanajima et al., 1998), or that we simply did not have sufficient subjects to demonstrate statistically significant facilitation. If so, then a simplified conclusion would be that cTBS had an inhibitory effect on the circuits underlying MEP production (I wave circuits), SICI and ICF, while iTBS had an opposite effect on these circuits.

We found our different TBS paradigms to have large effect sizes and acceptable inter-individual variability compared with traditional rTMS paradigms. Thus, the mean percentage change of MEP size in the period where the maximum effect occurred (i.e. 7-14 min after cTBS300, 15-40 min

after cTBS600, 1-10 min after iTBS) was -45.0% (SD= 8.9%), –42.2% (SD=24.0%) and 75.7% (SD=40.9%), respectively. These effect sizes and variability compare well with traditional rTMS paradigms, such as those explored by Maeda et al (2000), where a much larger number of rTMS pulses (1600) produced mean effects of –34.03% (SD=37.87%) after 1Hz and 37.87% (SD=53.59%) after 10Hz.

The effectiveness of these paradigms raises ethical issues about the use of these methods in normal human subjects, who have nothing to gain from modulation of synaptic plasticity, in contrast to patients with particular neurological disorders. We were aware of these ethical issues, and as well as putting our proposed experimental methods before the ethics committee of our institution and gaining consent from subjects, we pursued the experiments in an incremental fashion starting with smaller intensities and lower frequencies of stimulation than those reported here. We found in all experiments that cortical excitability eventually returned to baseline, and no subjects reported any side effects from experimentation. However, as methods for inducing plastic changes in human cortex become more powerful, such issues will require constant scrutiny and vigilance on the part of experimenters.

The results of the experiments with single trains of TBS suggest that in humans TBS produces a mixture of facilitatory and inhibitory effects on synaptic transmission, with facilitation building up faster than inhibition. If we assume that both facilitation and inhibition saturate at some level, then it is possible to explain the main features of the results as long as we allow inhibition to dominate in the long run. Thus, a short, intermittent protocol such as iTBS would favour rapid build up of facilitation. In contrast, a longer lasting continuous protocol such as cTBS would initially produce facilitation, but evenetually this would saturate and inhibitory effects which build up slower, but saturate at a higher level would dominate. An intermediate protocol such as imTBS might have no net effect by achieving a balance between the build up of inhibitory and facilitatory effects. This model is speculative at this stage, but would be consistent with several studies in animal preparations in which a mixture of opposing effects on LTP and LTD has been induced by the same protocol. For example, blocking some of the pathways that are needed for LTD induction, e.g. inositol triphosphate receptors (Nishiyama et al., 2000), can result in LTP after a protocol that usually produces LTD, whereas blocking LTP-dependent receptors, e.g. NMDA subunit 2A (Liu et al., 2004), may convert LTP into LTD. In addition, it has been shown that on occasion, a single protocol can cause LTP in some neurons whereas it results in LTD in others (Barbosa et al., 1990; Blackstone et al., 2003).

In conclusion, we have developed novel methods of delivering rTMS based on patterns of theta burst stimulation. We have found these stimulation paradigms to be safe in normal subjects, and capable of producing consistent, rapid and controllable electrophysiological and behavioural changes in the function of the human motor system that outlast the period of stimulation by over 60 minutes. In particular we have found that the pattern of delivery of TBS (continuous versus intermittent) is crucial in determining the direction of change in synaptic efficiency. The method may prove useful not only in the motor cortex but also in other regions of the brain for both the study of normal human physiology and for therapeutic manipulation of brain plasticity.

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APPENDIX 2: PUBLICATIONS AND ABSTRACTS ARISING FROM WORK PERFORMED DURING PhD PERIOD.

PUBLICATIONS

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