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TEMPORAL DISCRIMINATION THRESHOLDS AS AN ENDOPHENOTYPE

IN

ADULT-ONSET PRIMARY TORSION DYSTONIA

DR. DAVID BRADLEY MB BCH BAO MRCPI

DECLARATION

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ABSTRACT

Adult onset primary torsion dystonia (AOPTD) is the most common form of dystonia and is an autosomal dominant disorder with markedly reduced penetrance. Most AOPTD patients therefore present as "sporadic" cases. Disordered sensory processing is found in AOPTD patients; if also present in their unaffected relatives this abnormality may indicate non-manifesting gene carriage (an endophenotype). A sensitive endophenotype would be particularly helpful in ongoing and as yet unproductive efforts to discover the gene or genes involved in AOPTD. The Temporal Discrimination Threshold is the shortest time interval at which two stimuli can be detected to be asynchronous. Temporal discrimination thresholds are abnormal in a number of disorders involving basal ganglia dysfunction, but their utility as a possible AOPTD endophenotype has not been examined. A number of experiments were carried out to explore the validity of the temporal discrimination threshold as an endophenotype in adult onset primary torsion dystonia.

The frequency of abnormal temporal discrimination thresholds was examined in sporadic and familial AOPTD patients, their unaffected first degree and second degree relatives and healthy control subjects using visual and electrical stimuli for two (visual and tactile) or three (visual, tactile and mixed) tasks. The relative frequencies of abnormal temporal discrimination thresholds in patients, relatives and controls were compatible with an autosomal dominant endophenotype. Inheritance patterns in AOPTD pedigrees also provide strong evidence for the role of TDT as an endophenotype.

Structural MRI (Voxel-based Morphometry; VBM) was undertaken in unaffected relatives of AOPTD patients and demonstrated a disease-associated phenomenon (putaminal

enlargement) in relatives with abnormal TDTs, validating the endophenotype and indicating that this abnormality is likely a primary feature of dystonia gene carriage.

The temporal discrimination threshold was compared to a previous candidate endophenotype - spatial discrimination threshold (SDT) – and other published measures. This indicated that TDT is more sensitive and specific than other currently available methods.

The characteristics of the temporal discrimination threshold test were examined by comparing three task types and testing different AOPTD phenotypes. The multimodal mixed TDT task was shown to be less sensitive than the unimodal tasks and similar frequencies of abnormalities were seen across AOPTD phenotypes.

The temporal discrimination threshold was examined in sporadic AOPTD patients and their first degree unaffected relatives. This strongly supports the hypothesis that sporadic patients are the only manifesting individuals of a poorly penetrant gene or genes in their family.

Functional MRI scanning was used to compare AOPTD patients, unaffected relatives and control subjects during TDT testing and demonstrated that normal temporal discrimination is an index of putaminal function in AOPTD. Abnormal temporal discrimination in relatives of patients with AOPTD performing a TDT task was associated with functional hypoactivation of the putamen; this study further validates the TDT as an endophenotype in AOPTD.

The aims of this thesis were completed, and the findings taken together provide convincing evidence that TDT is a robust, sensitive and specific marker of non-manifesting dystonia

gene carriage, identifying subclinical (predominantly basal ganglia) dysfunction in affected patients and an appropriate percentage of unaffected relatives. While not specific to the basal ganglia dysfunction seen in AOPTD, when correctly applied the method may prove extremely useful in AOPTD genetic studies.

PUBLICATIONS

Bradley D, Whelan R, Walsh R, Reilly R, Hutchinson S, Molloy F, Hutchinson M. **Temporal Discrimination Threshold: VBM evidence for an endophenotype in adult onset primary torsion dystonia.** *Brain 2009: 132(9); 2327*

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Bradley D, Whelan R, Walsh R, O'Dwyer J,Reilly R, Hutchinson S, Molloy F, Hutchinson M. **Comparing Endophenotypes in Adult-Onset Primary Torsion Dystonia.** *Mov Disord 2010: 25(1); 84*

Bradley D, Whelan R, Kimmich O, O'Riordan S, Mulrooney N, Brady P, et al., Temporal **Discrimination Thresholds in Adult-Onset Primary Torsion Dystonia: an analysis by task type and by dystonia phenotype.** *J Neurol 2011 (DOI 10.1007/s00415-011-6125-7)*

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D Bradley, S O'Riordan, M Hutchinson. **Familial adolescent onset scoliosis and segmental dystonia.** *ENJ 2010:2(2);87-90*

Walsh R, O'Dwyer JP, O'Riordan S, Bradley D, Moroney J, Hutchinson M. **Cervical dystonia presenting as a phenocopy in an Irish SCA2 family.** *Mov Disord 2009: 24(3); 466*

PLATFORM PRESENTATIONS

Bradley D[1], Whelan R[1], Walsh R[1], Reilly R[2], Hutchinson M[1]. **The temporal discrimination threshold in patients with adult onset primary torsion dystonia and their unaffected relatives: a new endophenotype?** *[1]Dept of Neurology, St Vincent's University Hospital, [2]Dept of Neural Engineering, Trinity College Dublin.*

[ABN Dublin 2008]

Bradley D[1], Whelan R[1], Walsh R[1], Reilly R[2], Hutchinson M[1]. **Temporal Discrimination Threshold (TDT) and Spatial Discrimination Threshold (SDT) – comparing endophenotypes in Adult-onset Primary Torsion Dystonia (AOPTD).** *[1]Dept of Neurology, St Vincent's University Hospital, [2]Dept of Neural Engineering, Trinity College Dublin.*

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Bradley D[1], Whelan R[1,2], Walsh R[1], Reilly R[2], [1]Hutchinson S, [3]Molloy F, Hutchinson M[1]. **Temporal Discrimination Threshold (TDT) and Spatial Discrimination Threshold (SDT) – comparing endophenotypes in Adult-onset Primary Torsion Dystonia (AOPTD).** *[1]Dept of Neurology, St Vincent's University Hospital, [2]Trinity Centre for Bioengineering, [1]Department of Clinical Neurophysiology, Beaumont Hospital, Dublin, Ireland.*

[Dystonia Federation European Congress, Hamburg 2008]

R Whelan[1], D Bradley[1], R Walsh[1], RB Reilly[2], S Hutchinson[1], F Molloy[3], M Hutchinson[1]. **Temporal Discrimination Thresholds in AOPTD – Voxel Based Morphometry in unaffected relatives validates a new endophenotype.** *[1]Dept. Neurology, St. Vincent's Hospital, [2]Trinity*

Centre for BioEngineering, Trinity College, [3]Department of Neurophysiology, Beaumont Hospital, Dublin, Ireland

[Winner, Young Investigator Competition, EFNS, Florence 2009]

D Bradley[1], R Whelan[1], R Walsh[1], RB Reilly[2], S Hutchinson[1], F Molloy[3], M Hutchinson[1].

Temporal Discrimination Thresholds in familial AOPTD Pedigrees – use of a new endophenotype. *[1]Dept. Neurology, St. Vincent's Hospital, [2]Trinity Centre for BioEngineering, Trinity College, [3]Department of Neurophysiology, Beaumont Hospital, Dublin, Ireland*

[Winner, Harold Miller Prize, INA Dublin 2009]

POSTER PRESENTATIONS

Bradley D[1], Whelan R[1], Walsh R[1], Reilly R[2], Hutchinson M[1]. **Temporal Discrimination Threshold (TDT) and Spatial Discrimination Threshold (SDT) – Comparing Endophenotypes in AOPTD.** *[1]Dept. of Neurology, St. Vincent's University Hospital, [2]Dept of Neural Engineering, Trinity College Dublin.*

[Moderated Poster, Movement Disorders Society Congress, Chicago 2008]

PREAMBLE

Adult-Onset Primary Torsion Dystonia (AOPTD) is a common movement disorder known to have autosomal dominant inheritance with markedly reduced penetrance (in the region of 12-15%). Poor penetrance has made genetic study of the disorder difficult, and in general terms unsuccessful to date.

The endophenotype approach to addressing the problem of poor penetrance in genetic studies is an established method and as sensory symptoms and findings are common in individuals with AOPTD, they provide an easily accessible and often easy to measure marker that may allow assignment of gene status to non-manifesting carriers.

The temporal discrimination threshold is a sensory measure known to be abnormal in AOPTD as well as a number of other disorders. It is considered to be a marker of basal ganglia dysfunction in particular, and this likely is the reason for the apparent high prevalence of abnormal TDT results in patients with various forms of dystonia.

This thesis examined a cohort of familial and sporadic AOPTD patients, their first degree relatives and control subjects to assess the role of TDT as an endophenotype. This is achieved by examining the frequencies of abnormal results across all three groups, examining AOPTD pedigrees with confirmed familial transmission of the disorder, and investigating the structural and functional imaging findings associated with TDT, and a comparison of TDT to a previous candidate endophenotype (spatial discrimination threshold; SDT)

MAIN AIMS

1. To examine temporal discrimination thresholds (TDTs) in both sporadic and familial Adult-Onset Primary Torsion Dystonia patients, their unaffected relatives and healthy controls and determine potential usefulness as an endophenotype.
2. To assess the validity of TDTs as an endophenotype by correlation with structural and functional MRI findings.
3. To compare TDTs with spatial discrimination threshold testing and other proposed or published candidate endophenotypes in AOPTD

RESEARCH QUESTIONS

1. What is the normal temporal discrimination threshold in healthy control subjects?
2. What is the frequency of abnormal temporal discrimination thresholds amongst sporadic (no other family member affected) and familial (positive family history) patients with adult onset primary torsion dystonia?
3. What is the frequency of abnormal temporal discrimination thresholds amongst clinically unaffected relatives of both sporadic and familial adult onset primary torsion dystonia patients?
4. What is the pattern of TDT inheritance in familial adult onset primary torsion dystonia pedigrees?
5. Is the temporal discrimination threshold compatible with a useful endophenotype in adult-onset primary torsion dystonia?
6. Can a structural correlate of abnormal temporal discrimination thresholds be demonstrated using volumetric MRI in unaffected relatives of adult onset primary

torsion dystonia patients? What does this add to knowledge on the pathogenesis of adult onset primary torsion dystonia? Do the findings validate the endophenotype?

7. What does an analysis of the temporal discrimination threshold in sporadic adult onset primary torsion dystonia patients and their unaffected first degree relatives tell us about the likelihood that these cases are in fact all genetic with markedly poor penetrance? Is there any age or gender effect on transmission?
8. What differences can be demonstrated between adult onset primary torsion dystonia patients, unaffected relatives and healthy controls using functional MRI? What does this add to knowledge on the pathogenesis of the disorder and can a functional correlate of abnormal TDT be demonstrated amongst unaffected relatives? Do the findings validate the endophenotype?
9. How do temporal discrimination thresholds compare to spatial discrimination thresholds, a sensory test previously examined as a candidate endophenotype in the Department of Neurology at St. Vincent's University Hospital in terms of frequency of abnormalities amongst patients, relatives and controls?

CHAPTER 1
INTRODUCTION

Dystonia is a common movement disorder, generally defined as a "syndrome of sustained muscle contractions, frequently causing twisting and repetitive movements, or abnormal postures" (Fahn, 1988, Fahn *et al.*, 1987). The disorder was first definitively described in 1911 (Oppenheim, 1911) following review of four Jewish patients with dystonic features – namely muscle spasm with genetic transmission ("dystonia musculorum deformans"). Descriptions of movement disorders that may well have been dystonia predate this (Barraquer, 1897, Destarac, 1901, Hammond, 1890) and a detailed description of typical dystonic phenomenology appeared in a thesis by Schwalbe, when the syndrome was attributed to hysteria (Schwalbe, 1908).

Dystonia was generally considered a psychogenic disorder even well into the mid-20th century (Goetz, 2001) when psychotherapy was often the mainstay of management. As understanding of the disorder evolved, particularly through careful phenotyping of patients, observation of movement disorders induced by L-dopa therapy of Parkinson's disease, accurate recording of family history and the ongoing discoveries of imaging, neurophysiological and other features in patients, dystonia was eventually recognized to be a feature of a diverse group of movement disorders, many of which have a genetic basis.

THE CLASSIFICATION OF DYSTONIA

The classification of dystonia continues to evolve, reflecting the complex and heterogeneous nature of this set of disorders and ongoing progress in their understanding. Generally, dystonia is classified by age of onset (young onset or adult onset), affected body region

(focal, segmental in which two or more contiguous body parts are involved, or generalized) and aetiology (primary or secondary) (Fahn, 1988). Progress in genetics has added to this classification system (Bressman, 2004, Bressman *et al.*, 2000).

In general, primary dystonia patients have dystonic features without other significant neurological abnormalities apart from tremor or myoclonus. In addition, neuro-imaging should be normal (Table 1.1). These patients are classified by genotype, if known, or else by age of onset and body region affected (phenotype).

Genetically Determined Primary Dystonia		
Designation	***Transmission*** ***Gene*** ***Protein***	***Clinical Features***
DTY1 ("Early-Onset Generalised Dystonia")	Autosomal Dominant Chr 9q34 Torsin A	Lower-limb onset with subsequent generalization, variable phenotype, reduced penetrance (50%)
DYT2	Autosomal Recessive Unmapped Unknown Protein	Unconfirmed; early-onset segmental or generalised
DYT3 (X-Linked Dystonia-Parkinsonism, "Lubag")	X-Linked Recessive Xq13.1 TAF1/DYT3	Initially generalized dystonia, followed by L-Dopa unresponsive parkinsonism.
DYT4	Autosomal Dominant Unmapped Unknown Protein	Cervical and laryngeal (single family)
DYT6	Autosomal Dominant Chr 8p21-q22 THAP1	Cranial, laryngeal, with or without limb involvement
DYT7	Autosomal Dominant 18p11 Unknown Protein	Cervical predominantly, postural tremor
DYT13	Autosomal Dominant 1p36.13 Unknown Protein	Cranio-cervical

Table 1.1: *The known primary genetic dystonias by locus, pattern of inheritance, gene product, and phenotype.*

The presence of additional neurological or systemic features should lead to the suspicion of a secondary cause for the patient's presentation, including structural lesions (tumours or

trauma), stroke, or other acquired causes ("acquired" dystonia, table 1.2). Otherwise, secondary dystonia may be genetically determined, either with dystonia as the primary feature (the "dystonia-plus" syndromes, table 1.3) or as an associated feature (the "heredo-degenerative" dystonia, table 1.4).

Secondary (Acquired) Dystonia
Cerebral palsy
Trauma
Stroke,
Cerebral neoplasm/tumour
Multiple sclerosis
Encephalitis
Antiphospholipid syndrome
Medications
Toxins
Psychogenic

Table 1.2: *The causes of acquired (non-genetic) secondary dystonia.*

Dystonia-Plus, Paroxysmal and Related Dystonia Syndromes		
Designation	**Transmission, Gene, Protein**	**Clinical Features**
DYT5 / GCH1 (Dopa-Responsive Dystonia, "Segawa")	Autosomal Dominant 14q22.1-22.2 GTP cyclohydrolase 1	Incomplete penetrance. Variable dystonia responds to small doses of L-Dopa. Marked diurnal variation.
DYT8 / PNKD (non-kinesigenic dyskinesia, "Mount-Reback")	Autosomal Dominant 2q33-q35 Myofibrillogenesis regulator 1	Incomplete penetrance. Episodic childhood-onset dystonia with chorea and athetosis precipitated by alcohol, caffeine, stress.

Dystonia-Plus, Paroxysmal and Related Dystonia Syndromes		
Designation	**Transmission, Gene, Protein**	**Clinical Features**
DYT9 / CSE ("choreoathetosis/spasticity, episodic")	Autosomal Dominant 1p21 Unknown Protein	Episodic dystonia, spastic paraplegia, ataxia, parasthesia, diplopia. Precipitated by exercise, stress, alcohol.
DYT10 / PKC ("paroxysmal kinesigenic choreoathetosis")	Autosomal Dominant 16p11.2-q12.1 Unknown protein	Incomplete penetrance. Episodic dystonia and choreoathetosis triggered by movement
DYT11 (Myoclonus-Dystonia)	Autosomal Dominant 7q21-q23 Epsilon-sarcoglycan	Incomplete penetrance. Dystonia with myoclonus; sensitive, to alcohol
DYT12 (Rapid-onset dystonia parkinsonism)	Autosomal Dominant 19q12-13.2 ATP1A3 (Na/K ATPase α3 subunit)	Incomplete penetrance. Acute onset generalized dystonia with parkinsonism.
DTY14 (historical interest) (Dopa-responsive dystonia) ***Now known to be DYT5***	Autosomal Dominant 14q13 Initially unknown, now known to be GTP cyclohydrolase 1 (DYT5)	Single family with dopa-responsive dystonia,initially thought to be a new locus, repeat analysis showed a novel GTP cyclohydrolase 1 mutation (DYT5).
DYT15 (Myoclonus Dystonia)	Autosomal Dominant 18p11 Unknown protein	Myoclonus and/or dystonia

Table 1.3: *The genetically determined secondary dystonias; "dystonia-plus".*

Genetically Determined Secondary Dystonias	
Dystonia-Plus Syndromes (Disorders with prominent dystonia plus other neurological features)	X-linked dystonia Parkinsonism (Lubag; DYT3), Dopa – Responsive dystonia (DYT5) Myoclonus dystonia (DYT11) Rapid onset dystonia Parkinsonism (DYT12)
Degenerative Syndromes without Parkinsonism	*Autosomal Dominant*: Huntington's disease, spinocerebellar ataxias, dentatorubral-pallidolysian atrophy (DRPLA), fronto-temporal dementia, Huntington's disease-like 2, prion diseases *Autosomal Recessive*: Wilson's disease, pantothenate-kinase associated neurodegeneration (PANK), neuroacanthocytosis, Friedrich's, ataxia telangeictasia, ataxia with occulomotor apraxia, lysosomal disorder diseases *X-linked recessive*: Lesch-Nyhan Syndrome, Mohr-Tranebjaerg syndrome (deafness-dystonia) *Mitochondrial*: Myoclonic epilepsy with red ragged fibres (MERRF), mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), Leber's syndrome
Degenerative Parkinsonian Syndromes	Idiopathic Parkinson's Disease, Progressive Supranuclear Palsy, Corticobasal ganglionic degeneration, multiple systems atrophy. Familial Parkinson's Disease: PARK2 (Parkin mutations), LRRK2
Other Inherited Disorders	Paroxysmal non-kinesigenic choreoathetosis (DYT8) Paroxysmal choreoathetosis and ataxia (DYT9) Paroxysmal kinesigenic dyskinesia (DYT10)

Table 1.4: *The heredo-degenerative dystonias.*

ADULT-ONSET PRIMARY TORSION DYSTONIA

Adult-Onset Primary Torsion Dystonia (AOPTD) is the most common form of primary dystonia. AOPTD is a focal dystonia with onset usually after age 26 and without secondary causes. Spread of focal dystonia to adjacent anatomical sites may occur within five years of onset, described as segmental (for example a cervical dystonia patient may develop focal hand dystonia) but AOPTD never becomes generalised. There are several phenotypes according to the region affected including cervical dystonia, blepharospasm, focal hand dystonia, spasmodic dysphonia, oro-mandibular dystonia and Meige's Syndrome (the combination of blepharospasm and oro-mandibular dystonia) (Figure 1.1). In 25% of cases, more than one family member can be affected (Stojanovic *et al.*, 1995). Within such families the form of dystonia can vary and forme frustes can exist along with other neurological diagnoses, namely tremor.

Prevalence studies are difficult in AOPTD and estimated worldwide range from under 50 to over 7000 cases per million (Muller *et al.*, 2002, Nakashima *et al.*, 1995, Nutt *et al.*, 1988, Pekmezovic *et al.*, 2003). Adjusted estimates from the most robust studies provide figures of 600 per million (England) and 3,000 per million (Italy) within Europe (Defazio *et al.*, 2004). The estimated number of AOPTD patients in Ireland is 3,000-3,500. Cervical dystonia is the commonest form and is characterized by turning (torticollis) flexion (anterocollis), extension (retrocollis) or lateral flexion of the neck (Chan *et al.*, 1991, Fahn, 1984). It is commonly associated with shoulder elevation and this is considered by some to be segmental dystonia. The relative frequencies of AOPTD phenotypes varies; in the largest European study to date, a cross-sectional analysis in eight European countries revealed the relative frequencies to be cervical dystonia 57 per million, blepharospasm 36 per million, laryngeal dystonia 7 per

million, and limb dystonia 17 per million (EDSE Group, 2000). There is regional variation however, with the Northern England study revealing the prevalence in 372 patients to be cervical dystonia 61 per million, blepharospasm 30 per million, oromandicular dystonia 1 per million and laryngeal dystonia 8 per million (Duffey *et al.*, 1998). These results are in line with a German study in 188 patients showing cervical dystonia at 54 per million, blepharospasm at 31 per million, oromandibular dystonia 2 per million and laryngeal dystonia at 10 per million (Castelon Konkiewitz *et al.*, 2002) and an Icelandic study in 107 patients showing cervical dystonia 115 per million, blepharospasm 31 per million, oromandibular dystonia 28 per million and laryngeal dystonia 59 per million (Asgeirsson *et al.*, 2006). In contrast, a Spanish study in 48 patients reported relatively more prevalent blepharospasm, with cervical dystonia at 75 per million, blepharospasm 102 per million and laryngeal dystonia 2 per million (Duarte *et al.*, 1999). A summary of European AOPTD prevalence studies, including further studies in Norway (Le *et al.*, 2003), Italy (Papantonio *et al.*, 2009), and Belgrade (Pekmezovic *et al.*, 2003), is shown in table 1.5

Study *Location*	No of Patients	Gender Ratio (F:M)	Age at Onset	Type	Prevalence (per million)
EDSE, 2000 *Europe; 8 Countries*	879	1.3	46.6	***Overall*** Cervical Bleph OMD Laryngeal Limb	***117*** 57 36 -- 7 14
Duffey et al, 1988 *Northern England*	372	2.4	42.4	***Overall*** Cervical Bleph OMD Laryngeal Limb	***113*** 61 30 1 8 12
Castelon Konkiewitz et al, 2002 *Germany*	188	1.5	50.4	***Overall*** Cervical Bleph OMD Laryngeal Limb	***101*** 54 31 2 10 4
Asgeirsson et al, 2005 *Iceland*	107	1.8	41.9	***Overall*** Cervical Bleph OMD Laryngeal Limb	***312*** 115 31 28 59 80
Duarte et al, 1999 *Spain*	48	n/a	48.4	***Overall*** Cervical Bleph OMD Laryngeal	***286*** 75 102 89 20
Le et al, 2003 *Norway*	129	2.1	46.3	***Overall*** Cervical Bleph OMD Laryngeal	***254*** 130 47 8 28

Study *Location*	No of Patients	Gender Ratio (F:M)	Age at Onset	Type	Prevalence (per million)
Pekmezovic et al, 2003 *Belgrade*	165	1.5	46.0	***Overall*** Cervical Bleph OMD Laryngeal	117 59 19 3 11
Papantonio et al, 2009 *Italy*	69	1.4		***Overall*** Cervical Bleph Other	***127*** 44 68 15

Table 1.5: *Prevalence rates reported by European epidemiological studies in AOPTD patients, along with reported gender ratio mean age at onset.*

Type of Dystonia	Illustration	Clinical Features	Differential diagnosis
Cervical Dystonia (Spasmodic Torticollis) - most common focal dystonia		Initially neck stiffness and restricted head mobility. Abnormal head postures follow, sometimes with irregular head tremor. Neck and shoulder pain in 75% Onset usually between 30 and 50 years of age	• Essential head tremor • Tardive dyskinesia • Cervical myopathy or multiple system atrophy • Secondary torticollis associated with neck injury, atlantoaxial dislocation, cervical disease, spinal cord neoplasm or soft tissue infection of the neck
Cranial Dystonia (including Blepharospasm)		May involve eyelids, jaw, vocal cords, face, tongue, platysma or pharynx. Blepharospasm is the commonest – increased blink frequency, forced eye closure or difficulty opening eyes.	• Tic disorders • Ptosis (Myastenia gravis) • Dry eyes • Secondary blepharospasm can be caused by Parkinson's disease, tardive dyskinesia or dry eyes
Oromandibular dystonia		Involuntary clenching, opening, or deviation of the jaw. Muscles of mouth, tongue neck are also frequently involved. Severe cases cause jaw pain, dysarthria, difficulty chewing, dysphagia	• Temporomandibular joint disorders • Bruxism • Edentulous mouth movements • Tardive dyskinesia
Limb dystonia (including writer's cramp)		Involves the arm less frequently than the leg. Involuntary twisting flexion or extension postures of the arms legs or digits. Occurs in association with skilled manual activities – occupational cramp disorders.	• Orthopaedic overuse syndromes. • Nerve entrapment. • Muscle cramps.

Figure 1.1: *The common AOPTD phenotypes; Adapted from (Tarsy and Simon, 2006).*

THE PATHOPHYSIOLOGY OF AOPTD

Our understanding of the underlying patho-physiological mechanisms in AOPTD remains incomplete, although ongoing work has revealed a multitude of not only motor but also sensory and other abnormalities in these patients. Aberrant neurotransmission and electro-physiological processes underlie deficient inhibition and abnormal plasticity but the precise interaction of these observations remains elusive. The discovery of dystonia genes, while helpful, has not yet resulted in a comprehensive model for dystonia. There appear to be specific processes in some of the genetic forms of dystonia that result in eventual expression of dystonia with specific features. For example, in DYT1 dystonia (Torsin A) and DYT11 (Myoclonus Dystonia) (epsilon-sarocoglycan), the relevant proteins are likely involved in neural trafficking and in DYT5 the affected GTP Cyclohydrolase 1 is involved in neurotransmission but the phenotypes manifested are often indistinguishable from the those present in idiopathic forms of generalized dystonia. A reasonable suspect in the pathogenesis of AOPTD is basal ganglia dysfunction, which is implicated in other hyperkinetic movement disorders, for example Huntington's disease. However, clearly this is not a complete explanation and a summary of current knowledge of the pathology of AOPTD (which is common to most forms of dystonia) is presented. In particular, basal ganglia abnormalities and sensori-motor integration deficits are consistent findings. Dystonia patients have characteristic findings that can be obtained using standard electrophysiological measurement. These include co-contraction of agonist and antagonist muscles, tremor, lack of fine motor control, impairment of volitional muscle contraction and prolonged muscle fibre electrical discharges. These findings have long been clinically appreciated (Cohen and Hallett, 1988) and all suggest impaired inhibition in motor control.

EFFECT OF AGE AT ONSET

There is an association between age of onset and site of dystonia that has long been recognized (Denny-Brown, 1968, Marsden, 1976). In fact, onset after age 26 (in any family member) essentially excludes a DYT1 dystonia for which genetic testing is available (Bressman, 2004). In an early dystonia cohort study, the mean age of onset in patients who eventually developed established generalized, segmental or focal dystonia was 9, 30 and 41 years respectively (Marsden *et al.*, 1976). In a larger series, similar findings were seen in 560 patients with the mean age of onset in generalized, segmental and focal dystonia being 8, 40 and 45 years (Fahn, 1986).

Within focal dystonia, there is an effect of age of onset on the phenotype. In an early series findings mean ages of onset in blepharospasm, oromandibular dystonia, writer's cramp and cervical dystonia were 57, 56, 43 and 42 years respectively (Marsden *et al.*, 1976). Similar findings were described in 1988 in a series from Minnesota with the mean age of onset in blepharospasm (56 years) and oromandibular dystonia (66 years) exceeding that of cervical dystonia (45 years) or writer's cramp (49 years) while those with generalized dystonia had a mean onset at 20 years (Nutt *et al.*, 1988). A meta-analysis performed in the Department of Neurology at St. Vincent's University Hospital found the mean ages to be: DYT1 dystonia 11 years, writer's cramp 39 years, cervical dystonia 41 years and blepharospasm/OMD 56 years (O'Riordan *et al.*, 2004).

In addition, phenotype variation is reported within families with multiple AOPTD patients (Bressman *et al.*, 1994, Holmgren *et al.*, 1995, Micheli *et al.*, 1994, Munchau *et al.*, 2000, Uitti and Maraganore, 1993) and in our Department 4 of 12 identified families

demonstrated phenotypic variation (O'Riordan, 2006). Similar findings are reported in eight families in a review of AOPTD aetiological factors (Defazio *et al.*, 2007). In the same review, a meta-analysis of pairs of affected first-degree relatives with AOPTD showed that 38 pairs were concordant by phenotype and 33 pairs had differing phenotypes. While AOPTD may be genetically heterogenous, it seems probable that the same genetic disorder in these reported families and pairs of relatives results in different AOPTD phenotypes.

One may hypothesize, therefore, that it is more likely that age at onset modulates the phenotype expressed in genetically determined dystonia rather than the alternative hypothesis that different dystonias simply present at different ages. One suggestion is that causative dystonia genes may exert a more disabling and generalized effect on an immature nervous system (Marsden *et al.*, 1976). Other factors have been implicated, for example gender and sex hormones (Soland *et al.*, 1996) and even ApoE genotype (Matsumoto *et al.*, 2003). Somatotopy is recognized in the putamen (Gerardin *et al.*, 2003, Maillard *et al.*, 2000) and given the importance of the putamen in AOPTD pathogenesis, one may also hypothesize that age related changes in this region influence the somatotopic expression of symptomatic dystonia genes.

NEUROTRANSMISSION

Normal Basal Ganglia Function: Our concept of the role of the basal ganglia in normal and abnormal movement has evolved over greater than a century with successive lesional, animal and post-mortem analyses contributing to models of how the basal ganglia integrate cortical and peripheral information to modulate motor function.

The prevailing model of basal ganglia function (Figure 1.2) crystallised in the 1980s based on some seminal work (Albin *et al.*, 1989, DeLong, 1990, Penny and Young, 1983) and proposes that the basal ganglia process information from multiple brain regions. This originates from two primary observations;

1. Anatomical and neurochemical evidence (Albin *et al.*, 1989, Alexander *et al.*, 1986, DeLong, 1990) suggested that different groups of striatal medium spiny neurons (MSNs) project either to
    a) the *medial* globus pallidus pars interna (MGP or GPi) and substantia nigra pars reticulata (SNr) via monosynaptic connections exerting an inhibitory and phasic effect via GABA. These MSNs express D1, Substance P and dynorphin receptors. This is the ***direct pathway***.
    b) The GPi and SNr eventually through a polysynaptic relay involving MSN → *lateral* Globus Palludis pars externa (LGP or GPe) →Subthalamic Nucleus (STN) → GPi exerting an excitatory effect via glutamine. These MSNs express D2 and encephalin receptors. This is the ***indirect pathway***.
2. Physiological data suggested that the output from the GPi/SNr exerts tonic inhibitory control on thalamic and brainstem structures (the ***rate model***), with movement (e.g. saccadic eye movements) facilitated by brief pauses in this tone (Chevalier and Deniau, 1990). Therefore, activation of the direct pathway (which inhibits basal ganglia output) facilitates movement and activation of the indirect pathway (which excites basal ganglia output) inhibits movement. There is experimental evidence that with dopaminergic depletion (as seen in Parkinson's Disease), there is downregulation of D1 expression in the direct pathway activity and upregulation of

D2 expression indirect pathway activity, explaining the bradykinesia and rigidity seen in the condition (Gerfen *et al.*, 1990). The success of subthalmotomy in treating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism in primates further supported this hypothesis (Aziz *et al.*, 1991, Guridi *et al.*, 1996) and led to surgical therapies and more recently deep brain stimulation for Parkinson's disease and related conditions. Conversely, the hypothesis was also proposed that hyperkinetic movement (dyskinesia or hemiballism/chorea) may be accounted for by impairment of GPi output (Crossman, 1990).

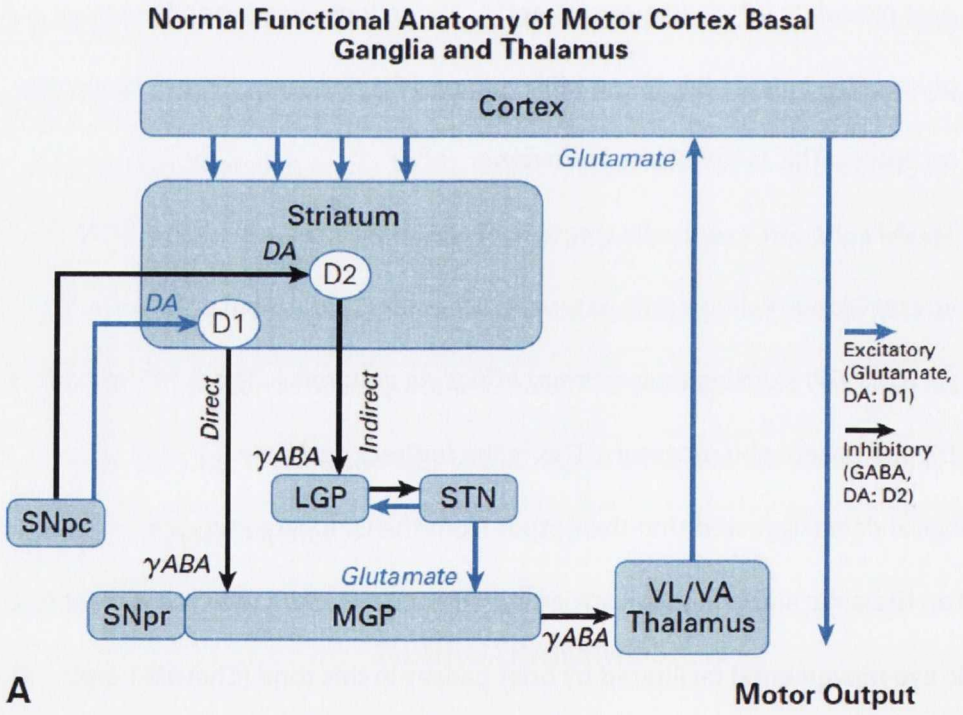


Figure 1.2: *Schematic drawing of the classical basal ganglia circuits, from (Ropper and Brown R, 2005). Blue lines excitatory; black lines inhibitory. LGP = Lateral Globus Pallidus (same as the Globus Pallidus pars externa (GPe)); MGP = Medial Globus Pallidus (same as Globus Pallidus pars interna (GPi)); SNpr = Substantia Nigra pars reticulata complex; STN = Subthalamic Nucleus; SNpc = Substantia Nigra pars compacta; VA/VL Thalamus = ventrolateral and ventroanterior nuclei of thalamus.*

The model is not without inconsistencies however, including the fact that GPi lesions can abolish dyskinesia (as opposed to allow excessive movement due to impaired basal ganglia inhibitory output) (Obeso *et al.*, 1997) and furthermore the fact that lesions in the motor thalamus do not exacerbate Parkinson's Disease (Marsden and Obeso, 1994). The recent finding that basal ganglia output is hypersynchronised in Parkinson's Disease (Brown, 2003, Brown *et al.*, 2001) may explain the benefit of GPi lesioning in dyskinesia (ablating abnormal hypersynchrony) and it appears that motor thalamic lesions can in fact result in subtle abnormalities in motor learning (Redgrave *et al.*, 2010).

Recent advances in basal ganglia physiology: In recent years, a number of findings have updated the original model described above (Figure 1.3). The STN is now known to have much more extensive connections than previously defined, including input from the motor-related cortical areas (including the pre-supplementary motor area) (Inase *et al.*, 1999, Nambu *et al.*, 2000, Nambu *et al.*, 2002) and thalamus (Lanciego *et al.*, 2004). This cortical-subthalamic-pallidal connection has been termed the ***hyperdirect pathway***. In addition, the STN outputs to areas in the ventral thalamus (Rico *et al.*, 2010) and connections between the striatum and GPe (Sato *et al.*, 2000) as well as the striatum and SNc (Haber *et al.*, 2000). There is also the suggestion that a circuit exists between STN-GPe-GPi that affords additional control to the GPe over basal ganglia output (Obeso *et al.*, 2006). Furthermore, input to the basal ganglia is now known to originate from the superior colliculus, locus coeruleus, raphe nuclei, caudal intralaminar nuclei, pedunculopontine nucleus and thalamus in addition to the cortex (McHaffie *et al.*, 2005).

The recent understanding of the importance of interneuronal function in the striatum also aids our appreciation of basal ganglia function. The two main populations of concern are the

tonically active interneurons (TANs) (Cholinergic) and the Fast Spiking Interneurons (FSIs) (GABAergic). Both result in inhibitory effects, the TANs through presynaptic inhibition of excitatory cortical (glumatatergic) input to the MSN and the FSIs through feed forward inhibition (Bonsi *et al.*, 2011, Tepper *et al.*, 2010). These interneurons likely provide the ability to focus and select basal ganglia activity.

Finally, the role of the dopaminergic system in basal ganglia function has been expanded. Dopamine innervates several structures outside the striatum (Smith and Villalba, 2008), including the STN, GPi, GPe, cortex, thalamus and limbic structures (***"nigro-extrastriatal" pathway***). Dopaminergic pathways are divided a medio-ventral and a dorso-lateral projection and the majority of dopaminergic innervation consists of a widespread tonic effect on striatal activation (Moss and Bolam, 2008). In addition, however, there is a more focal effect on phasic SNc synaptic firing that may allow motor learning through feedback of expected versus actual reward/outcome (Bromberg-Martin *et al.*, 2010). Furthermore, there is now evidence that less than 10% of MSNs may co-express D1 and D2 receptors, in contrast to the commonly held figure of approx. 50% (Bertran-Gonzalez *et al.*, 2010).

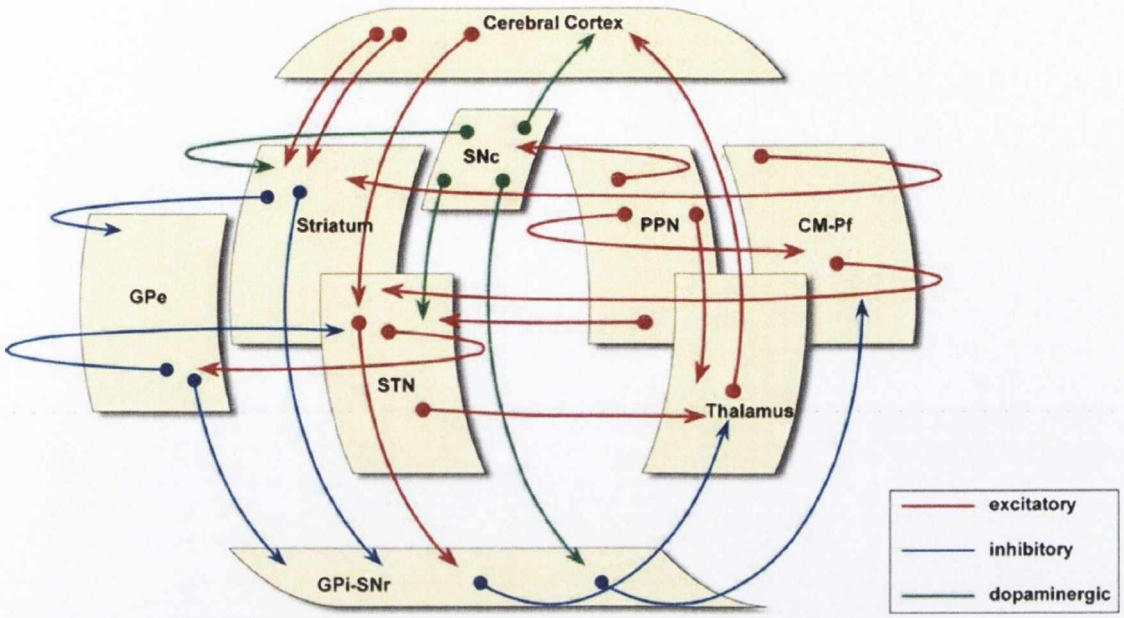


Figure 1.3: *Updated schematic of basal ganglia function, illustrating additional connections not considered in the classical direct/indirect pathway model, taken from (Obeso and Lanciego, 2011). GPe = Globus Pallidus pars externa; GPi-SNr = Globus Pallidus pars interna/Substantia Nigra pars reticulata complex; PPN = pedunculopontine nucleus; STN = Subthalamic Nucleus; SNc = Substantia Nigra pars compacta.*

Dopamine: This neurotransmitter is perhaps the most consistently linked to the pathophysiology of dystonia, and indeed movement disorders in general. Tardive dystonia and acute dystonic reactions are specifically related to dopaminergic blockade (specifically D2) while dopa-responsive dystonia is exquisitely sensitive to dopamine replacement. The ability of drugs like tetrabenzine, which deplete dopamine through impairment of its storage, to relieve tardive dystonia implies also that dopamine is required to sustain dystonic symptoms. Dystonia is common in dopamine-deficient conditions, in particular Parkinson's disease and related disorders. These clinical observations aptly demonstrate the fundamental role for dopamine in dystonia.

Reduced D2 receptor (D2R) availability in dystonia: D2R availability is reduced in both manifesting patients and non-manifesting carriers in both DYT1 and DYT6 dystonia (Carbon et al., 2009). Reduced D2R availability has been shown previously in DYT1 dystonia (Asanuma *et al.*, 2005), in cranial dystonia (Perlmutter *et al.*, 1997) and cervical dystonia (Naumann *et al.*, 1998). The reduction is not severe, approximately 30% in all studies in both affected and non-affected gene carriers. Reduced D2R availability would result in reduced inhibitory effect of dopamine on the medium spiny neurons (MSN) of the striato-pallidal indirect pathway to the external globus pallidum (GPe), resulting in disinhibited thalamo-cortical output and excessive dystonic movements.

D2 receptors have been examined in a number of imaging studies of primary dystonia. Decreased binding in imaging may reflect receptor loss or altered synaptic dopamine levels. Reduced binding to D2 in the putamen has been demonstrated using various agents (Naumann *et al.*, 1998, Perlmutter *et al.*, 1997). Non-manifesting DYT1 carriers also have decreased D2 uptake in caudate and putamen (Asanuma *et al.*, 2005) indicating either a subclinical primary gene effect (and potential endophenotype) or a compensatory response, but not a causative role. Presynaptic dysfunction has been demonstrated in familial idiopathic dystonia (Playford *et al.*, 1993) and abnormalities in dopamine (and metabolites) are known in autopsy series of dystonia patients (Furukawa *et al.*, 2000, Hornykiewicz *et al.*, 1986).

In the mouse model of DYT1, there is evidence that mutant *torsinA* results in deficient long term depolarisation (LTD) and synaptic depotentiation (SD), along with enhanced long term potentiation (Martella *et al.*, 2009). Furthermore, aberrant D2 function (important in LTD)

can be restored by adenosine A2A receptor antagonism (Quartarone and Pisani, 2011); these receptors are co-localised with D2 receptors and antagonise their effects.

Acetylcholine: Cholinergic antagonists are often used clinically with good effect in generalized and focal dystonia. While a direct role for acetylcholine is not defined, this neurotransmitter interacts with several transmitters and neural circuits. In particular, there seems to be an interaction with dopamine; for example dopaminergic afferents have a strong influence on cholinergic interneuron transmission in the striatum (Pisani *et al.*, 2007). Furthermore, as described above, tonic cholinergic interneurons likely have a role in movement selection in the striatum; while this cholinergic population in the striatum is small, these fibres are tonically active and exert an effect on GABAergic neurons, possibly modulating responses to cortical and thalamic input (Tepper and Bolam, 2004). In the mouse model of DYT1 (with mutant *TorsinA*), lowering acetylcholine levels or the use of an M1 antagonist both restored subnormal LTD and SD (Martella *et al.*, 2009). In the same study, enhanced cholinergic tone was reported in the DYT1 mouse, evidenced by elevated acetylcholinesterase activity.

GABA: The role of this neurotransmitter in both CNS inhibitory pathways and synaptic plasticity is relevant in considering its place in dystonia. GABAergic drugs, such as benzodiazepines, have a clinical utility in some patients with dystonia. In a novel study using a new technique (J-resolved protein multiple-metabolite spectroscopy) to measure cortical and striatal GABA, reduced levels were seen cortically and subcortically contra-lateral to the affected side in focal hand dystonia patients (Levy and Hallett, 2002) and in animal studies, GABA blockade results in some dystonic features, including co-contraction (Matsumura *et al.*, 1991).

DISINHIBITION

Lack of the normal inhibition required to allow fine control of motor output is held to be a cardinal feature of dystonia (Hallett, 2010) and is described in the cortex, striatum and brainstem. The fundamental principle is that lack of suppression of adjacent or antagonist muscles in voluntary motor activity results in overflow, co-contraction and dystonic muscle activity. An early study showed that patients with spasmodic torticollis had impaired exteroceptive suppression in the sternomastoid muscle following supra-orbital nerve stimulation, indicating impairment of interneuronal inhibitory pathways (in this case between the 5^{th} and 11^{th} nerves) (Nakashima *et al.*, 1989). A further study showed abnormal excitation of facial motor neurons during a study of the blink reflex in patients with blepharospasm and oromandibular dystonia (with a normal reflex arc), postulated to arise from the basal ganglia (Berardelli *et al.*, 1985). These abnormalities may be the basis for abnormal co-contraction of agonist and antagonist muscles seen commonly in dystonia. In the central nervous system, cortical inhibition has been shown to be abnormal in several studies. Inhibitory function in the cortex can be assessed using transcranial magnetic stimulation (TMS) and a "double pulse" experimental paradigm that delivers an initial "conditioning" stimulus (subthreshold) followed by an active (suprathreshold) stimulus designed to activate descending pathways. The conditioning pulse usually results in inhibition so that the supra-threshold pulse, that should normally result in muscle activity, results in no EMG activity in the target muscle. Failure of production of this normal effect suggests reduced intracortical inhibition. In writer's cramp patients, bilateral reduction in intracortical inhibition has been demonstrated initially in 1995 (Ridding *et al.*, 1995). Intracranial facilitation was found to be normal. Others have confirmed this pattern and

replicated impaired cortical and subcotical inhibition in dystonia (Ceballos-Baumann and Brooks, 1997, Ceballos-Baumann *et al.*, 1995, Huang *et al.*, 2010, Ibanez *et al.*, 1999, Levy and Hallett, 2002, McDonnell *et al.*, 2007). It is hypothesized that failure of the normal activation of cortical GABAergic inhibitory neurons underlies these findings i.e. GABA interneurons are dysfunctional in dystonia. Abnormal intracortical silent periods are associated with disinhibition; the silent period is due to an initial refractory period in the spinal cord followed by intracortical inhibition. The silent period is shortened in dystonia (Chen *et al.*, 1997). Again, these features do not appear to be directly causative of dystonia but are nonetheless widespread in the CNS of dystonia patients.

PLASTICITY

Plasticity is conceptualised as a feature of the nervous system whereby function (effectiveness of transmission) of established neural circuits can change over time. Mechanisms include alterations in membrane thresholds, synaptic transmission or receptor expression. Plasticity plays an important role in memory and recovery after nervous system injury but abnormal plasticity may also lead to neurological disorder. One hypothesis is that abnormal plasticity results in disease in susceptible individuals following triggers that normally should result in useful cortical adaptation (skill practice or injury).

The concept of abnormal plasticity/motor learning is a common theme in dystonia patho-physiological investigation (Hallett, 2006). Changes in plasticity may relate both to disinhibition and abnormal sensori-motor integration and one suggestion is that at least some of the motor features seen in dystonia relate to abnormal sensory input and re-modelling of the motor structures in response. It is known that skilled repetitive motor

activity, for example in highly practiced musicians, or even highly repetitive generic activity, for example in clerks before the advent of typewriters and other systems, may result in dystonia that persists even after cessation of the inciting activity. A seminal animal study induced dystonic features acutely in monkeys trained in repeated specific actions (Byl *et al.*, 1996). It was demonstrated in these animals that there was disorganization in the somatosensory cortex with markedly enlarged and overlapping digit representations and receptive fields in the primary sensory cortex. An elegant study increased our understanding of plasticity by examining plasticity in rats following basal ganglia lesioning (Schicatano *et al.*, 1997). In this study, weakening of the obicularis oculi muscle in normal rats resulted in an adaptive gain of function (plasticity-related increase in drive), allowing eye closure function to be regained. This normal plastic effect was abnormally exaggerated in rats with a pre-existing lesion (striatal dopamine depletion which resulted in tonic inhibition of the blink reflex at baseline), and resulted in a blepharospasm phenotype. This indicated that the an underlying subclinical abnormality (basal ganglia dysfunction) can cause abnormal motor manifestations in a situation where plasticity was recruited. Transcranial magnetic stimulation can be used to demonstrate this using an experimental paradigms of paired associative stimulation (in which peripheral stimulation of, for example, the median nerve is coupled to a TMS impulse to the sensori-motor area) and repetitive TMS (rTMS). The principle is that TMS stimulates firing of pyramidal cortical neurons, and therefore descending pathways, by activating neurons that synapse on them; they are not stimulated directly. Therefore, the magnitude of CNS response is a marker of the excitability of this network of neurons, rather than the pyramidal neurons themselves. Low frequency TMS (1Hz) tends to suppress motor evoked potentials whereas high frequency TMS (5Hz) potentiates them; these provide a model of long term depression and long term

potentiation respectively. These effects persist after application representing the induction of plastic changes.

There are numerous studies indicating that the plasticity seen during normal motor learning is abnormal in dystonia, and in particular that sensorimotor plasticity appears to be enhanced; an early study demonstrated that the rTMS response in focal hand dystonia exceeded that of controls (Siebner *et al.*, 2003). This implied enhanced plasticity with the caveat that the observed changes may alternatively relate to differences in the resting state of the premotor cortex. Further work using paired associative stimulation (PAS) addressed this (Quartarone *et al.*, 2003): when an ascending stimulus (from the median nerve) was timed to arrive at the motor cortex 25ms before TMS was applied, the magnitude of the motor evoked potential (MEP) in that specific somatotopic region was enhanced (facilitated) with an increase in cortical silent periods (due to increased excitability of inhibitory interneurons) in controls. This represented normal plastic changes. In dystonia patients, this MEP facilitation was more pronounced, the somatotopic specificity was lost and the expected increase in silent period was absent, providing direct evidence for enhanced plasticity in dystonia. Negative (depressive) plastic changes have also been demonstrated to be more readily elicited in dystonia patients (Baumer *et al.*, 2007). In a study of blepharospasm patients, another method (EMG measurement of blink reflex following unilateral high frequency supra-orbital nerve stimulation) again demonstrated enhanced plasticity in response to stimulation (Quartarone *et al.*, 2006).

The control and balance of plasticity in the CNS appears to be of critical importance, allowing the acquisition of new learned motor skills and memory function without resulting in unwanted, excessive or abnormal circuit development and enhancement. However, the

positive feedback element of plasticity carries the risk of establishment and progression of abnormal circuit function (Turrigiano and Nelson, 2000). The concept of "homeostatic plasticity" (Bienenstock *et al.*, 1982) is relevant in this regard and holds that a form of auto-regulation occurs in neural plasticity: in situations with significant neural postsynaptic activity and feedback, long-term potentiation processes are inhibited and the converse occurs in the case of diminished neural activity, thus preventing significant detrimental alteration in neural circuits. In a study aimed at examining this, subjects were pre-conditioned with 10 minutes of transcranial direct current stimulation (to represent increase in neural activity) prior to using a standard TMS procedure to assess plasticity (Siebner *et al.*, 2004). When low frequency TMS was applied to controls, an expected increase of an inhibitory LTD-type effect was seen as would be predicted by homeostatic plasticity theory. In patients with focal hand dystonia, however, this did not occur: rTMS could not overcome the increased excitability caused by transcranial direct current stimulation. This implies that the auto-regulatory process that keeps plasticity within limits is impaired in these patient and this is a conceivable mechanism for the aberrant circuits seen in these patients.

Again the question arises as to whether abnormal plasticity is causative in whole or in part or whether it is an epiphenomenon or secondary change. Furthermore, the question arises as to whether homeostatic plasticity has an effect in practical terms. Studies in healthy controls examining the effect of learning a motor skill (representing preconditioning) on plasticity (as measured by response to TMS) found that the principles of homeostatic plasticity held (Stefan *et al.*, 2006, Ziemann *et al.*, 2004): the learning of a task impaired LTP type plasticity (measured by facilitatory PAS) and enhanced LTD type plasticity (measured using inhibitory PAS).

As before, impaired plasticity may be thought of as an endophenotype that interacts with other physiological abnormalities: for example GABA is likely important in plasticity and is abnormal in these individuals. Sensory retraining techniques may have a significant role to play in dystonia management given the demonstrable effects on plasticity in healthy control subjects as described above.

MOTOR CIRCUITS

The Basal Ganglia: This is a natural site to consider in the pathogenesis of dystonia given the convergence of peripheral and central sensory and motor findings found. Animal studies commonly demonstrate that lesions in the putamen and globus pallidus result in dystonia. There are several imaging studies that demonstrate basal ganglia abnormalities in these patients. For example, reduced D2 binding in the putamen has been demonstrated in focal dystonia (Chase *et al.*, 1988) and DYT1 dystonia (Asanuma *et al.*, 2005). In addition, resting state functional MRI has demonstrated abnormal lentiform nucleus activity in dystonia (Eidelberg *et al.*, 1998) with increased excitability demonstrated in this region also (Blood *et al.*, 2004). Bilateral putaminal enlargement, as indicated by bilateral increase in grey matter density using voxel-based morphometry (VBM) has been demonstrated in focal dystonia patients (Etgen *et al.*, 2006) and this is explored in unaffected relatives in the study described in chapter three. Further structural abnormalities, namely abnormal fractional anisotrophy using diffusion tensor imaging, has been demonstrated in the lentiform nucleus (Colosimo *et al.*, 2005). These findings all implicate the basal ganglia, the putamen in particular, as integral to the pathophysiology of dystonia.

The common element of dystonia pathogenesis is abnormal focusing of motor activity; excitatory and inhibitory imbalance and dysfunction lead to abnormal and poorly focused motor output with overflow and co-activation of agonist and antagonist muscles. The inability of the basal ganglia to perform their required function likely reflects a combination of neurotransmitter dysfunction, abnormal plasticity and/or structural abnormalities in this region.

Further abnormalities are evident in most other areas related to motor function. Lesions in such structures as the caudate, cerebellum, brainstem or thalamus produce dystonia in animal studies. There is evidence of physiological abnormality in many regions throughout the CNS motor system structures not localized to affected body regions. These observations indicate, therefore, that a single motor structure is unlikely to be the underlying cause of dystonia but rather that the disorder is one of global motor dysfunction.

The Cerebellum: Cerebellar pathology is associated with dystonia, with multiple lesional case reports published a significant association in one secondary dystonia case series (LeDoux and Brady, 2003). Posterior fossa tumours are associated with a cervical dystonia phanotype (Extremera *et al.*, 2008). Structural imaging findings are variable, with both increased and decreased grey matter density reported (Delmaire *et al.*, 2007, Draganski *et al.*, 2003, Obermann *et al.*, 2007). PET imaging studies tend to report increased metabolic activity (Eidelberg *et al.*, 1998, Eidelberg *et al.*, 1995, Niethammer *et al.*, 2011) and fMRI studies reveal disordered cerebellar activation (Baker *et al.*, 2003, Hu *et al.*, 2006, Kadota *et al.*, 2010, Preibisch *et al.*, 2001, Simonyan and Ludlow, 2011, Wu *et al.*, 2010), interestingly inversely related to disease severity in some, possible reflecting a compensatory role. Electrophysiological abnormalities of cerebellar function are also well documented, for

example using the eye blink classical conditioning (EDCC) paradigm (Teo *et al.*, 2009). There is certainly compelling evidence for aberrant cerebellar function, primary or secondary, in dystonia; absence of clinical evidence of cerebellar dysfunction in patients with primary dystonia implies a compensatory role but investigation of this area is as yet preliminary.

SENSORY ABNORMALITIES

Aside from abnormalities in motor function, sensory pathology is well recognized in dystonia. The most clinically obvious example is the "geste antagoniste" present in a proportion of patients. Examples include touching the chin or ear (thereby providing tactile or proprioceptive input) in order to temporarily relieve dystonia symptoms (Muller *et al.*, 2001). Patients with blepharospasm may complain of sensory symptoms (gritty eyes) and increased symptoms in strong light and patients with laryngeal dystonia may present with pharyngeal pain (Ghika *et al.*, 1993). Some patients also report significant pain as part of their dystonia (Pekmezovic *et al.*, 2009). One hypothesis is that sensory dysfunction may interfere with the control of motor functions either due to incorrect sensory input or inappropriate handling of sensory feedback from peripheral structures, for example the muscle spindle, and that this contributes to the pathogenesis of dystonic movement.

A number of sensory abnormalities are detectable in patients with dystonia. Measurable psycho-physical tasks such as the spatial and temporal discrimination threshold are discussed in more detail later in this Thesis. In addition, abnormal vibration-induced illusion of movement is reported in dystonia patients and relatives (Frima *et al.*, 2008, Frima *et al.*, 2003, Rome and Grunewald, 1999, Rome and Grunewald, 2000). The fact that vibration may worsen focal hand dystonia features further supports the idea that abnormal muscle spindle

function may be a component of dystonia pathogenesis (Kaji *et al.*, 1995). Importantly, Kaji's study also demonstrated that the dystonic features induced by vibration in patients (not seen in controls) could be abolished by "de-afferentation"; they injected lidocaine into hyperactive muscles which reduced spindle function (as indicated by decreased tendon reflexes) with little effect on the M-response, indicating that it was the reduction in spindle afferent transmission that helped dystonic features. This effect was not seen with injection of non-dystonic muscles. Abnormality of movement representation is likely an important factor: aberrant sensory feedback may interfere with accurate and efficient motor planning and execution. The basal ganglia, along with the parietal and visual cortices and further areas including the pre-motor cortex, supplementary motor area and motor cortex form a network in which sensory input and planning are mapped onto motor output and impairment of this sensori-motor integration appears to be a fundamental feature of dystonia. The mental rotation paradigm has been studied as a model of abnormal movement representation and has demonstrated abnormalities in both cervical dystonia and writer's cramp patients (Fiorio *et al.*, 2006, Fiorio *et al.*, 2007). Interestingly, the abnormality was more localized in the focal hand dystonia study, suggesting local factors are more important in that phenotype.

The finding of generalized abnormalities in sensory function in patients with focal dystonia imply a generalized CNS disorder with superimposed triggers resulting in the expression of disease. The presence of some of these abnormalities in non-manifesting carriers of dystonia genes, for example DYT1 (discussed later) indicate subclinical presence of these features as a risk factor or endophenotype in a susceptible host. The critical role of the basal ganglia in sensory function is evidenced by the fact that abnormalities in spatial and

temporal processing as well as proprioception and movement representation are also seen in other movement disorders in which the basal ganglia are affected (Amick *et al.*, 2006, Artieda *et al.*, 1992, Dominey *et al.*, 1995, Duncombe *et al.*, 1994, Helmich *et al.*, 2007, Lee *et al.*, 1998, Maschke *et al.*, 2005, Sathian *et al.*, 1997, Zia *et al.*, 2000). A table with sensory and other non-motor features in primary dystonia is presented in Table1.6 , taken from a recent extensive review (Stamelou *et al.*, 2011).

Primary dystonia	Sensory abnormalities				Neuropsychiatric abnormalities	
Adult-onset primary dystonia	Temporal discrimination (higher TDT)	Spatial discrimination (impaired SDT)	Vibration (impaired VIIM)	Impaired mental rotation task	Risk for Anxiety (higher)	Risk for Depression (higher)
Blepharospasm	Yes (Fiorio 2008; Scontrini 2009; Bradley 2011)	Yes (Molloy 2003; Walsh 2007; Walsh 2009)	Yes (Grunewald 1997; Yoneda 2000)	not tested	No (Fabbrin 2010)	Yes (39,3% vs. 4% HC (Fabbrini 2010))
Focal hand dystonia	Yes (Bara-Jimenez 2000; Tamura 2008; Bradley 2009)	Yes (Bara-Jimenez 2000; Bradley 2010; Molloy 2003)	Yes (Frima 2003; Rome 1999; Putzki 2006)	Yes (Fiorio 2006)	No (Fabbrini 2010)	No (Fabbrini 2010)
Writer's cramp	Yes (Sanger 2001; Fiorio 2003; Scontrini 2009)	Yes (Sanger 2001; Bradley 2010; Bara-Jimenez 2000)	Yes (Yoneda 2000)	Yes (affected and unaffected hand but not the feet; Fiorio 2006)	No (Fabbrini 2010) Yes (Gundel 2001; Lencer 2009)	insufficiently tested
Cervical dystonia	Yes (Tinazzi 2004; Scontrini 2009; Bradley 2009; 2010; 2011)	Yes (Bradley 2010; Molloy 2003)	Yes (Yoneda 2000)	Yes (head, hand and feet; Fiorio 2007)	No (Fabbrini 2010)	Yes (26.4% vs. 6% HC (Fabbrini 2010) 17.95%, no control group (Voon 2010))
Laryngeal dystonia	Yes (Scontrini 2009; Bradley 2009; 2011)	insufficiently tested (Walsh 2009)	not tested	not tested	No (Fabbrini 2010)	Yes (14.6% vs. 3.7% (Gundel 2007)) No (Fabbrini 2010)
Generalized	Yes (Tinazzi 2002; Aglioti 2003)	insufficiently tested (Walsh 2009)	not tested	not tested	not tested	not tested
Unaffected relatives	Yes in first and second degree (CD, WC, FHD) (Bradley 2009; 2010; 2011)	Yes 24% first and second degree (FHD) (O'Dwyer 2005) Yes, 50% first degree (CD, WC) (Bradley 2010)	Yes 60% first degree (CD) (Frima 2008)	not tested	not tested	Family history of depression in 41,02% of FHD, no control group (Voon 2010)
DYT1 dystonia						
manifesting carriers	Yes, tactile, visuotactile (vs. non-carriers) (Fiorio 2007)	No (Molloy 2003)	not tested	Yes (Fiorio 2008)	No (Heiman 2007)	Yes (Heiman 2004)
non-manifesting carriers	Yes, tactile, visuotactile (vs. non-carriers) (Fiorio 2007)	not tested	not tested	Yes (Fiorio 2008)	No (Heiman 2007)	Yes (Heiman 2004)

Figure 1.6: *The non-motor features of dystonia, taken from a recent review (Stamelou et al., 2011).*

GENETICS OF AOPTD

Despite convincing evidence for a genetic basis for AOPTD (significantly more prevalent than early-onset primary dystonia for which many genes are mapped), efforts to uncover genes associated with AOPTD have been generally unsuccessful to date. The majority of AOPTD cases that present clinically have no family history and appear to be sporadic. However, detailed investigation of the family will reveal at least one further individual with an AOPTD phenotype in up to 25% of cases (Waddy *et al.*, 1991). In fact, asking the proband whether a family history exists during consultation has a sensitivity of only 27% with a specificity of 98% (Martino *et al.*, 2004). One alternative, apart from formally examining the family of a patient, seems to be the use of a standardized, computer assisted telephone interview method (Aniello *et al.*, 2006).

Epidemiological studies provide evidence that AOPTD is transmitted in an autosomal-dominant fashion (Leube *et al.*, 1997, Stojanovic *et al.*, 1995) with markedly reduced penetrance (in the region of 12%-15%). A common hypothesis is that all of these cases are genetically determined and the poor penetrance results in the high frequency of apparently sporadic presentations. This poor penetrance is the main reason for the difficulty in elucidating AOPTD genes to date and the endophenotype approach to addressing this problem is the main theme of this thesis.

There has been significant progress in the determination of the genetic basis of several types of dystonia. The seminal example is DYT1, a GAG mutation in the TOR1A gene on chromosome 9q that results in an aberrant *TorsinA* protein (Kramer *et al.*, 1988, Ozelius *et al.*, 1989, Ozelius *et al.*, 1997). This results in an early-onset (before age 26), limb-onset

dystonia that becomes generalized in two thirds of cases (Bressman *et al.*, 2000). There are frequent developments in the discovery of specific genetic dystonia syndromes. An important recent example that may prove to be the specific genetic basis for some AOPTD patients is DYT6: mutations in THAP1 resulting in an early-onset cranial (commonly laryngeal) focal dystonia syndrome (Bressman *et al.*, 2009, Djarmati *et al.*, 2009, Fuchs *et al.*, 2009). Prior to gene localisation, the previously recognised and rare DYT6 phenotype had been mapped in families that included some AOPTD patients, in a similar fashion to other phenotypes including DYT7 (18p) and DYT13 (Almasy *et al.*, 1997, Leube *et al.*, 1996, Valente *et al.*, 2001). Of interest, the probands from 6 large multiplex families from the Department of Neurology at St. Vincent's University Hospital were screened and none were found to be DYT6 carriers (unpublished data).

Despite the number of identified monogenic dystonias (Muller, 2009), the genetic causes of most AOPTD phenotypes remain unknown. The low penetrance of the disease makes genetic studies (e.g. linkage analysis) difficult as it is not possible to create two homogenous comparison groups (gene carriers and non-gene carriers). One approach to this problem is the use of an endophenotype (see below).

TREATMENT OF DYSTONIA

The management of AOPTD depends on a number of factors. Treatment generally consists of pharmacotherapy in the form of medication or intramuscular botulinum toxin. Non-pharmacological options exist in the form of deep brain stimulation and in some forms of dystonia sensory retraining or physiotherapy approaches may be of benefit.

BOTULINUM TOXIN

Intramuscular botulinum toxin (BTX) produces weakness in injected muscles that can be of benefit in focal torsion dystonia in particular when correctly targeted. The therapeutic application of botulinum toxin was postulated as far back as 1820, when botulism was clinically described (Kerner, 1820), and a clinical preparation was first used in the 1980s in the treatment of strabismus (Scott, 2004). The mechanism involves inhibition of acetylcholine release, and therefore neuromuscular transmission, by interfering with the mechanisms required for Ach exocytosis, thereby causing "chemodene rvation".

Molecular Structure: Six botulinum toxin serotypes exist (A-F), eash comprising 150kD neurotoxin component with a protein complex of variable size which differs between serotypes and bacterial strains. The toxic and therapeutically active neurotoxin moiety consists of light and heavy chains forming a single polypeptide chain and linked by a highly conserved disulphide bond (S-S). The heavy chain directs BTX to its target: specific acceptor proteins on the extracellular domain of the presynaptic membrane at neuromuscular and autonomic postganglionic synapses. BTX is then internalised via endocytosis and the active 50kD light chain is cleaved by proteolysis from the inactive 100kD heavy chain by cleavage of the S-S bond and passes into the cytosol. The light chain is a zinc-dependant protease (metalloprotease) which cleaves one or more of the soluable NSF [N ethylmaleimide-sensitive fusion] attachment protein receptors (SNARE) proteins. Normally, SNARE proteins transport acetylcholine-containing vesicles from the cytosol to the synaptic cleft. Different serotypes of botulinum toxin interfere with different SNARE proteins. BTX types A, C and E cleave a synaptosomal associated protein of 25 kD (SNAP-25), each at a unique site.

Serotypes B, D and F cleave vesicle-associated membrane protein or VAMP, again each acting at a particular site.

The result is a failure of neurotransmitter exocytosis as acetylcholine vesicles cannot fuse with the presynaptic membrane to allow discharge. This leads to functional denervation of associated muscle or glandular structures. The term 'chemodenervation' is criticised by some as contact between the nerve terminal and target is not lost but temporarily inactivated and there is no motor axonal loss apparent. In response to BTX treatment, affected axons undergo axonal 'sprouting' whereby immature axons develop from the motor end plate and the nodes of Ranvier, likely in response the local release of growth factors. Acetylcholine receptors and acetylcholinesterase spread from the neuromuscular junction to other regions of the muscle plasma membrane. It was originally thought that this was the mechanism by which motor unit function was restored but it has been shown that these axonal sprouts die back once the original motor end plates resume normal function with the normal quantity of acetylcholine receptors and acetylcholinesterase being found exclusively at the junctions (de Paiva *et al.*, 1999). Botulinum toxin therefore does not appear to have any long-term anatomical or physiological consequences at the neuromuscular junction in that its effect is fully reversible. Induced weakness typically lasts 2 to 3 months but for autonomic disorders the therapeutic effect can last from six months to one year and the reason for this is not clear. At the neuromuscular junction there is some variation in the duration of action of each serotype of botulinum toxin with BTX-A having the longest lasting inhibitory effect. The reasons why particular serotypes might vary in their length of action may include (1) the lifetime of the light-chain in the cytosol, (2) the ability of

the neuron to regenerate new SNARE proteins and (3) the secondary biochemical effect of the production of truncated SNARE proteins.

Clinical Use in Dystonia: Botulinum toxin specifically targets striatal and smooth muscle fibres through its effect at the motor end plate. In injected muscles, atrophy and weakness become apparent within two weeks of treatment and arise as a consequence of the action of the zinc-endopeptidase within presynaptic membranes destroying proteins involved in process of acetylcholine release; hypertrophic dystonic muscle returns to normal volume. There is now direct evidence from animal studies and indirect evidence from human subjects to support the hypothesis that BTX has an additional effect on the central nervous system above and beyond that observed peripherally (Abbruzzese and Berardelli, 2006, Byrnes *et al.*, 1998, Curra *et al.*, 2004, Giladi, 1997, Priori *et al.*, 1995, Trompetto *et al.*, 2006, Walsh and Hutchinson, 2007). In keeping with this hypothesis, there are a number of observations which are not explained by a process taking place exclusively at the α-motor neuron end-plate: (a) Symptomatic improvement is at times out of proportion to induced muscle weakness, with reasonable strength maintained during voluntary muscle contractions. In addition, weakness alone would not be expected to reduce the frequency of dystonic movements, as is often reported by patients. Conversely, dystonic movements may remain unchanged despite the presence of marked atrophy and paralysis, (b) the therapeutic effect is not restricted to injected muscle groups or even surrounding regional muscles which may be affected by local toxin diffusion, (c) a dose-response curve exists with a correlation between reduced maximum EMG amplitude in affected muscle and dose. However, maximum decrements in EMG activity are seen at relatively small doses despite definite clinical improvement continuing as treatment dose increases suggesting a possible

therapeutic effect beyond that impacting on neuromuscular transmission, (d) in AOPTD phenocopies, botulinum toxin will not always produce similar responses, (e) Patients with post traumatic or tardive dystonia will often respond poorly to treatment. This suggests that the therapeutic outcome may rely on more than just the action of BTX on overactive muscle fibres but also one affecting a pathophysiological process specific to primary torsion dystonia.

Two types exist in clinical practice; type A (approved for use in the late 1980s, brand names "Botox" and "Dysport") and type B (brand name "Neurobloc"). Treatment is given every 3-6 months depending on patient response. As stated above, effect of therapy typically takes 5-10 days to manifest and the duration of effect ranges from typically 10 weeks to 6 months in a few cases. The major complications that occur with the agent are:

- Injection site pain or allergy
- Excessive weakness in the target muscle (e.g. excessive hand weakness in a writer's cramp patient) that interferes with function
- Excessive weakness in non-injected adjacent muscles (e.g. speech or swallow impairment in cervical dystonia patients. This is related to excessive dose, injection into an unrecovered muscle (still strophic from previous dose) or inaccurate injection site.
- Rarely, generalized weakness related to excessive total dosing, for example treatments more frequent than the recommenced 12-weekly interval

Cochrane reviews support the use of both serotypes in the treatment of cervical dystonia (Costa *et al.*, 2005, Costa *et al.*, 2005) with little to choose between them, although possibly

more dysphagia and xerostomia associated with type B (Comella *et al.*, 2005). Several studies also support the use of botulinum toxin in other forms of focal dystonia. The use of botulinum toxin in generalized dystonia is not typical, unless symptomatic relief in a particular body part (for example limb contracture) is required.

PHARMACOTHERAPY

After botulinum toxin, oral medications are the most common agents used in the management of dystonia (table 1.7). Several agents are utilized and will be considered briefly here.

Category	Agent	Typical Daily Dose (mg)
Anticholinergic	Trihexphenidyl	6-40
	Benztropine	4-15
	Ethoporpazine	100-400
Benzodiazepine	Lorazepam	1-6
	Diazepam	10-60
	Clonazepan	1-4
GABA Agnoist	Baclofen	40-120
Dopamine Precursor	L-Dopa (with Carbidopa)	75-600
Monoamine Depletor	Tetrabenzine	50-200

Table 1.7: *Common pharmacotherapies used in the treatment of dystonia.*

ANTICHOLINERGICS

Cholinergic antagonist drugs, for example trihexphenidyl, are well established in the management of hyperkinetic movement disorders. The benefit of therapy has been demonstrated in both open label (Fahn, 1983) and double-blinded (Burke and Fahn, 1983, Burke *et al.*, 1986) studies. Typically there is improvement in 60% of children and 40% of adults with dystonia when high doses are achieved. The mechanism of action is not completely understood. The therapeutic benefit of these agents is frequently limited by side effects. These include

- Peripheral: Constipation, dry mouth, urinary retention, blurred vision (these may be amenable to treatment with a peripheral anti-cholinesterase agent, such as pyridostigmine).
- Central: Confusion, restlessness, memory impairment, hallucinations or even frank psychosis.

Anticholinergic side effects are more commonly dose-limiting in adults, and trihexphenidyl is the agent of choice in these patients.

GABAERGIC AGENTS

The lack of cortical inhibition characteristic of dystonia is commonly attributed to basal ganglia dysfunction and potentiation of GABAergic inhibitory output from the basal ganglia is an established therapeutic approach in these patients.

Benzodiazepines: These agents enhance GABA binding to target receptors, which facilitates calcium influx and reduced neuronal firing.

Baclofen: This agent is an agonist at the pre-synaptic $GABA_{\beta}$ receptor at the spinal cord level. Children are more likely to respond than adults, and shorter disease duration predicts superior response. Baclofen has been studied in generalized dystonia (Greene and Fahn, 1992), including DYT1 dystonia (Anca *et al.*, 2003), where improvement in gait was particularly noted. Baclofen has also been studied in focal dystonia with variable results; in one series only 18% of cranial dystonia sufferers derived benefit. Side effects include sedation, hypotension, bladder dysfunction, dry mouth, ataxia, cardiovascular and respiratory depression. If these effects are dose limiting, the drug can be administered intra-thecally.

Intrathecal Baclofen: This treatment involves direct administration of the drug to the subarachnoid space using an infusion pump. The main advantage is achieving high therapeutic levels without disabling side effects. A test dose is typically administered to the lumbar sac prior to committing to pump tube insertion. The therapeutic benefit is variable as reported by clinical trials. In one series, only 2 of 14 patients benefited (Walker *et al.*, 2000) while in another 92% of 77 subjects (predominantly with cerebral palsy) had sustained benefit (Albright *et al.*, 2001). This is in line with the finding that patients with secondary dystonia tend to benefit more from this therapy. Patients with tardive dystonia (Dressler *et al.*, 1997) and dystonia with spasticity (Ford *et al.*, 1996) may particularly benefit. It is also commonly reported that lower limb symptoms respond better to this treatment, probably as a result of the site of infusion. Complications include the standard baclofen toxic effects (nausea, paraesthesia, bladder urgency, hypotension, respiratory distress) as well as equipment-related problems (CSF leak or CNS infection).

DOPAMINE MODULATORS

Basal ganglia dysfunction is a hallmark of dystonia, with evidence for imbalance between an overactive direct pathway and an underactive indirect pathway. Patients may derive benefit from dopamine augmenting or depleting drugs, but not both, and the mechanism for this remains unclear.

L-Dopa: Dopa-response dystonia, a specific genetically-determined form of dystonia, is exquisitely sensitive to minimal doses of levodopa, often with a dramatic and persistent benefit at doses well below those required for Parkinson's disease, for example. In patient with early onset dystonia, a trial of L-Dopa is mandatory as response to the drug produces an almost curative effect. Patients with other forms of dystonia may also have some response to L-Dopa, typically at larger doses. For example. in one series of 41 patients that failed to respond to anticholinergics, 5 had some response to L-Dopa (Greene *et al.*, 1988).

Tetrabenzine: This dopamine-depleting drug appears to be particularly useful in tardive dystonia. An open label study found one quarter of patients responded to dopamine depletion (Greene *et al.*, 1988). As noradrenaline and serotonin are also depleted, depression is a significant adverse event. Others include sedation, hypotension and parkinsonism. Although side effects are often dose-limiting, some patients achieve critical improvement in tardive dystonia and otherwise difficult to treat oromandibular dystonia.

Other anti-dopaminergic drugs: Drugs that block dopamine receptors, specifically typical and atypical neuroleptic agents, have been examined in dystonia. Rates of response vary; An open label study found one third of 26 patients had response to dopamine blockade (Greene *et al.*, 1988).Drugs that block the D2 receptor (including olanzapine, risperidone,

and primidone) cause extrapyramidal side effects, but quetiapine and clozapine, which have lower D2 affinity, may be more helpful. Clozapine use is limited by potentially life-threatening agranulocytosis (requiring monthly blood tests) but has shown benefit in tardive dystonia (Adityanjee and Estrera, 1996) and cranial dystonia (Karp *et al.*, 1999). Results in cervical dystonia were disappointing. Quetiapine is a safer option and has been tried in tardive dystonia (Sasaki *et al.*, 2004).

Miscellaneous Agents: Response to some anticonvulsants have been documented, for example 11% had moderate or greater benefit with carbamazepine in one study (Greene *et al.*, 1988). Levetiracteam has been used in Meige syndrome (Zesiewicz *et al.*, 2004) and generalized dystonia. Topiramate is often useful in dystonic tremor and focal hand dystonia and has been studied specifically in segmental dystonia (Papapetropoulos and Singer, 2006).

Lithium has been shown to be no better than placebo in a blinded trial (Koller and Biary, 1983) but has been reported useful in some generalized and cervical dystonia patients. Mexilitine and riluzole have been found to have benefit in small case series and alcohol-responsiveness is a feature of myoclonus-dystonia. Tizanidine, an agent often used instead of or in conjunction with baclofen in the treatment of spasticity, has not been found to be useful in cranial dystonia (Lang and Riley, 1992).

DEEP BRAIN STIMULATION

Despite initial use in the management of chronic pain, deep brain stimulation is currently an established therapy in the management of movement disorders, in particular Parkinson's

disease. In contrast to Parkinson's disease, where the subthalamic nucleus is typically stimulated, the internal globus pallidus is the usual target in dystonia patients.

The patients typically selected for DBS have primary generalized or segmental dystonia. Patients with both DYT1 and nonDYT1 primary dystonia appear to benefit most (Coubes *et al.*, 2004). Some forms of secondary dystonia may also benefit, for example tardive dystonia (Trottenberg *et al.*, 2005) or patothenate kinase-associated neurodegeneration (Castelnau *et al.*, 2005). The Canadian multicentre trial for deep brain stimulation in cervical dystonia patients reported that the procedure was well tolerated with improvements in disability, dystonia severity and depression scales (Kiss *et al.*, 2007). Other forms of focal dystonia have also been treated with DBS, for example Meige's syndrome (the combination of oromandibular dystonia and blepharospasm) (Houser and Waltz, 2005).

Deep brain stimulation is generally reserved for patients refractory to standard therapy due to the surgical risk attached and the requirement for relatively frequent review to monitor settings and replace the battery.

OTHER SURGICAL APPROACHES

Peripheral surgical techniques were used extensively in dystonia prior to the advent of botulinum toxin. Several techniques for selective peripheral denervation evolved although the preferred technique from the early 1990s onwards was the posterior ramisectomy (extradural sectioning of the dorsal rami) (Bertrand, 1993). A large series of 168 patients undergoing this procedure reported improvement of head position in 77% and pain in 81% of cases (Cohen-Gadol *et al.*, 2003). Other series have confirmed these findings with

patients generally tolerating the procedure well; side effects include dermatomal sensory disturbance or shoulder weakness.

Selective myectomy of neck muscles has also been employed in cervical dystonia patients either alone or in combination with denervation. Other techniques that have been attempted include sectioning of the anterior cervical roots in cervical dystonia patients, decompression of the spinal accessory nerve and selective sensory stimulation of the accessory nerve.

Other surgical interventions include Intrathecal baclofen pump insertion (see pharmacotherapy above) and spinal cord stimulation, which has been shown to be ineffective . Pallidotomy and thalamotomy have also been employed in dystonia treatment in the past; in a study of thalamotomy, 70% have improvement (25% significant) and 12% worsened (Cooper, 1976). Long term outcome with pallidotomy has been reported to be better (Yoshor *et al.*, 2001). In general, these ablative procedures have been abandoned due to adverse effects, including cognitive disturbance, weakness and bulbar dysfunction.

PHYSICAL/OCCUPATIONAL AND RELATED THERAPIES

These therapies generally aim to allow patients to compensate/adapt their motor function to minimize the impact of dystonia on day to day activities. Splints, braces and related devices play a role in patients with significant joint deformity to enhance function and, in conjunction with range of motion type exercises, to prevent contractures.

Focal dystonia may respond to specialized physical therapy techniques. Modest benefit has been shown in some patients with writer's cramp who wore a hand orthosis although the effect was lost immediately on removal of the device (Tas *et al.*, 2001).

Techniques such as "sensory motor retuning" have been applied in focal dystonia with some success. For example, patients with writer's cramp trained to read Braille found improvement in symptoms that in some cases persistent post therapy in addition to measurable improvement in spatial acuity as measured by spatial discrimination tasks (Zeuner *et al.*, 2002). Furthermore, there is evidence that these types of interventions may alter cortical representation of dystonic body parts, presumably via plasticity effects, that may result in ongoing benefit (Candia *et al.*, 2005). Sensory retraining following cessation of instrument use for a prolonged period (e.g. 6 months) is a common therapeutic approach in patients with various forms of musicians dystonia. Other methods have shown persistent benefit post treatment including repetitive transcranial magnetic stimulation (Siebner *et al.*, 1999) and transcutaneous electrical stimulation (Tinazzi *et al.*, 2005) although no large trials exist and the magnitude of benefit varies significantly.

In cervical dystonia, physiotherapy approaches have been studied, for example graded exercise programs with a relaxation technique, or electromyographic feedback (Jahanshahi *et al.*, 1991). Other techniques have been studied in small numbers, for example postural re-education (Smania *et al.*, 2003) or therapeutic muscle vibration (Karnath *et al.*, 2000) with varying success.

Recently, a paper reports the effect of retraining on magnetoencephaolgraphy (MEG) findings in a study of writer's cramp patients (2 groups – trained and untrained) and control

subjects; training the affected limb in writer's cramp patients (with a resulting clinical improvement) is associated with evidence of plasticity-related change. In the primary sensory cortex associated with the dystonic limb, the representations of digits 1, 2, 3 and 5 was similar in trained patients to that seen in healthy controls, while in the cortex associated with the non-dystonic (and non-trained) limb, the MEG findings were abnormal (enlarged and disorganised digit representation) similar to that seen bilaterally in the group of untrained writer's cramp patients (Bleton *et al.*, 2011). This supports the role of plasticity in compensation of dystonic symptoms and provides insight into the basis for the effect of sensorimotor retraining in these patients.

ENDOPHENOTYPES

An endophenotype may be considered to be a subclinical marker of genetic liability to a disorder, whether this is determined by carriage of a single gene mutation or a number of genetic risk factors. They are biomarkers (defined as any disease-associated biological finding) that fulfil a number of specific criteria which are designed to determine that the marker is associated with the presence of the gene rather than simply manifestation of the disease state; the endophenotype should be associated with the disease under investigation in the general population, a heritable trait transmitted with disease in pedigrees, a finding that is "state-independent" (i.e. unaffected by disease expression or treatment) and should have a higher frequency amongst unaffected relatives in pedigrees than in the general population. Examples of endophenotypes in the literature include laboratory measurements, such as copper studies in Wilson's disease, neurophysiological features, for

example the specific EEG findings in juvenile myoclonic epilepsy (Greenberg *et al.*, 1988),or imaging findings, the specific pattern of MRI white matter change in CADASIL (O'Sullivan *et al.*, 2001). Endophenotypes could be used in linkage studies to identify genetic loci in poorly penetrant disorders; a number of criteria for a proposed endophenotype exist (Gershon and Goldin, 1986, Gottesman and Gould, 2003, Leboyer *et al.*, 1998). An ideal endophenotype for an autosomal dominant disorder should be abnormal in all affected patients, half of unaffected first degree relatives and no control subjects.

Sensory abnormalities are well documented in AOPTD. The simplest and most clinically apparent example is the "geste antagoniste" or sensory trick present in a significant proportion of patients, a typical example being transient relaxation of abnormal movement in cervical dystonia on touching the chin. Measurable sensory abnormalities in AOPTD include abnormal spatial discrimination, temporal discrimination and vibration-induced illusion of movement (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). It is hypothesized that a disorder of sensorimotor integration, possibly involving the basal ganglia, is the cause of these sensory abnormalities. Given the evidence of a genetic disorder, there has been significant investigation of candidate sensory endophenotypes.

Endophenotypes have been particularly studied in DYT1 dystonia because of its incomplete penetrance, thus a potential endophenotypic trait can be validated in non-manifesting carriers of the GAG deletion in *TorsinA*. In addition, abnormalities demonstrated in non-manifesting DYT1 carriers (Carbon *et al.*, 2004, Carbon *et al.*, 2004, Eidelberg *et al.*, 1998, Fiorio *et al.*, 2007, Ghilardi *et al.*, 2003) support the hypothesis that the physiological abnormalities of sensory processing seen in dystonia result from genetic determinants

rather than secondary changes induced by the movement disorder. The characteristics of a useful endophenotype include:

- Must segregate with illness in the general population
- Must be heritable
- Must be state independent, manifesting whether illness is present or in remission
- Must co-segregate with the disorder within families
- Must be present at a higher rate within affected families than in the general population
- (Should be a characteristic that can be measured reliably, and be specific to the illness of interest)

THE TEMPORAL DISCRIMINATION THRESHOLD

The temporal discrimination threshold (TDT) is defined as the shortest time interval at which a subject can determine that two stimuli are asynchronous. Abnormal TDTs have been described in a number of conditions including DYT1-dystonia (Fiorio *et al.*, 2007), idiopathic dystonia (Aglioti *et al.*, 2003, Tinazzi *et al.*, 2002, Tinazzi *et al.*, 1999), writer's cramp (Bara-Jimenez *et al.*, 2000, Fiorio *et al.*, 2003, Sanger *et al.*, 2001, Scontrini *et al.*, 2009), blepharospasm (Fiorio *et al.*, 2008, Scontrini *et al.*, 2009), Parkinson's disease (Fiorio *et al.*, 2008, Lee *et al.*, 2005) and multiple system atrophy (Lyoo *et al.*, 2007) and as such may be an indicator of abnormal basal ganglia function rather than specific to any particular movement disorder. A table summarising the experimental findings of TDT testing in dystonia, taken from a recent extensive review (Stamelou *et al.*, 2011) is presented in Table 1.7.

References	Cohort	Stimulus	Temporal discrimination threshold	Correlation with motor impairment	Treatment
Bara-Jimenez, 2000	4 WC; 10 FHD; 13 healthy controls	Tactile	Patients: 96.7 ms versus healthy controls: 64.4 ms	No	The last three months no treatment; before that NA
Sanger, 2001	9 WC; 10 healthy controls	Tactile	Patients: 107 ms versus healthy controls: 46 ms	No	The last three months no treatment; before that NA
Tinazzi, 2002	8 Generalized; 1 WC; 1 segmental; 12 healthy controls	Tactile	Patients: 107.3 ms versus healthy controls: 35.7 ms	No	3 BT (6 months before); 2 anti-cholinergics; 5 none
Aglioti, 2003	8 Generalized; 10 healthy controls	Tactile; visual; visuotactile	Significantly higher in patients	Yes, with visuotactile stimuli	4 BT (4–5 months before); 2 anti-cholinergics; 2 none
Fiorio, 2003	14 WC; 13 healthy controls	Tactile; visual; visuotactile	Significantly higher in WC versus healthy controls in tactile and visuotactile No impairment in visual stimuli	Yes	8 None; 6 BT (6 months before)
Tinazzi, 2004	10 CD; 5 cervical pain; 10 healthy controls	Tactile; visual; visuotactile	Significantly higher in CD versus pain and healthy controls in tactile and visuo-tactile No impairment in visual stimuli	NA	8 BT (6 months before); 2 none
Fiorio, 2007	DYT1: 9 MC; 11 NMC; 9 NC; 11 healthy controls	Tactile; visual; visuotactile	Significantly higher in DYT1 MC and NMC carriers versus NC and healthy controls in tactile and visuotactile stimuli	No	3 Untreated; two BT (6 months before); 4 deep brain stimulation GPi
Fiorio, 2008a	19 BS; 19 HMS; 19 healthy controls	Tactile	Significantly higher in BS versus HMS versus healthy controls	No	All BT (5 months before)
Tamura, 2008	11 FHD; 11 healthy controls	Tactile	Significantly higher in FHD versus healthy controls	No	None BT (3 months before)
Scontrini, 2009	35 BS; 30 CD; 8 FHD; 9 LD; 35 healthy controls; 26 HMS	Tactile	Significantly higher in all three body regions–two affected and one unaffected in patients versus healthy controls	No	All BT (5 months before)
Bradley, 2009	20 CD; 13 FHD; 1 LD; 1 musician's dystonia; 42 first-degree relatives; 32 second-degree relatives	Tactile; visual	Significantly higher in 95% CD, 77% FHD; 52% first-degree relatives; 50% second-degree relatives	No	18 Patients, no statistical correlation between TDT and time since last injection (mean: 8.2 weeks)
Bradley, 2010	14 CD; 10 WC; 34 first degree unaffected relatives	Tactile; visual; visuotactile	Significantly higher to all stimuli in 83% of the patients and 41% of the first degree relatives	No	NA
Bradley, 2011	37 CD; 14 WC; 9 BS; 11 LD; 8 musician's dystonia	Tactile; visual; visuotactile	Significantly higher to all stimuli in 97.3% CD, 85.7% WC, 88.8% BS, 90.1% LD, 62.5% musicians, lower sensitivity of the visuotactile stimuli	NA	NA
Scontrini, 2011	24 CD versus healthy controls	Tactile	Significantly higher before and after 1 and 2 months botulinum toxin injections	No	TDT remained significantly higher before and after 1 and 2 months borulinum toxin injections

Figure 1.7: *The experimental evidence for TDT abnormalities in dystonia to date, taken from a recent review (Stamelou et al., 2011).*

Functional imaging studies have demonstrated activation of the basal ganglia and other subcortical structures during a TDT task; higher cortical activity specific to TDT (not seen in SDT testing) was found in the anterior cingulate and presupplementary motor area, these regions may be involved in the interpretation of timing information (Pastor *et al.*, 2004). In contrast, the basic timekeeper appears to be the putamen, where the earliest activation occurs during encoding of time intervals (Rao *et al.*, 2001). It has been demonstrated that easier TDT tasks induce greater putaminal activation than difficult TDT tasks (e.g. stimuli presented near the threshold for simultaneity perception) when additional areas are activated (Pastor *et al.*, 2008). In this way the putamen seems to act as the automatic time keeper, particularly in low-attention situations.

A study in Parkinson's disease examined the effect of dopaminergic medication on another measure of timing perception (coupled temporal memories) and found that in the "off" state, patients had impaired accuracy and precision while replicating time intervals (Malapani *et al.*, 1998). A more recent study examined Parkinson's disease patients and the effect of subthalamic nucleus deep brain stimulation and dopaminergic stimulation on TDT; they found that dopamine and not DBS resulted in improved results (Conte *et al.*, 2010). These data imply that temporal processing, including TDT, is influenced by dopaminergic pathways. In further studies, dopamine blockade impairs temporal discrimination ability with both haloperidol and the D2-selective remoxipride impairing function in experiments measuring in the range of in the 0.1 to 1 second range, but not at the millisecond range, suggesting different mechanisms (Rammsayer, 1990, Rammsayer and Classen, 1997, Rammsayer, 1999, Rammsayer, 1993).

Interestingly, a further Parkinson's disease study found that only TDT, and not age, UPDRS, finger bradykinesia score, or finger tapping task score, predicted poor performance on a coin rotation task score (an indicator of impaired manual dexterity) (Lee *et al.*, 2010) suggesting that impaired temporal processing at the millisecond level may in and of itself have clinical implications for the patient over and above the disorder for which it may be a marker.

The method of TDT measurement has an effect on results obtained and the importance of a standardized protocol for measurement across subjects is critically important. This is discussed in more detail in Chapter 2.

THE PHYSIOLOGY OF NORMAL TIME PERCEPTION

The ability of the brain to perceive and process sensory information is truly remarkable. The perception of time is a long-studied phenomenon, and the neural mechanisms underlying our ability to internally measure time intervals and latency are the subject of considerable research. Temporal processing draws on a number of cognitive domains including attention, working memory, and long term memory (Brown, 1997, Brown and Boltz, 2002, Taatgen *et al.*, 2007, Zakay and Block, 2004) and is affected by mood and emotion (Droit-Volet and Meck, 2007, Noulhiane *et al.*, 2007).

CONCEPTS IN THE MECHANISM OF TIME ENCODING

Mechanisms vary for different orders of magnitude of time duration: The cellular mechanisms of encoding time perception likely vary with duration, ranging from the suprachiasmatic nuclei and gene transcription regulation in circadian rhythms (Hinton and

Meck, 1997, King and Takahashi, 2000, Reppert and Weaver, 2002) to time-delayed excitatory and inhibitory axonal transmission in the millisecond range (Carr, 1993, Covey and Casseday, 1999), involved in such activities as speech recognition, music, motor modulation or sound localisation. Time processing in the range of seconds to minutes represents an important component of decision making and day-to-day activity, and has been demonstrated to occur in animals (Buhusi *et al.*, 2002, Drew *et al.*, 2005, Gribova *et al.*, 2002), and humans (Brannon *et al.*, 2004, Penney *et al.*, 2000, Rakitin *et al.*, 1998). This "interval" timing has been shown not to be related to suprachiasmatic nucleus function (Lewis *et al.*, 2003), but is shown to be impaired in individuals with cerebellar damage (Harrington *et al.*, 2004, Malapani *et al.*, 1998).

The Scalar Property: An interesting phenomenon in behavioural experiments on temporal processing is the scalar property; when participants are asked to reproduce a specified duration, their responses are distributed normally with the standard deviation proportional to the interval in question. When curves for different intervals are scaled, they overlap consistently i.e. the mean (specified interval in each case) and the standard deviation are correlated in a predictable manner (Gibbon *et al.*, 1984, Rakitin *et al.*, 1998). This is interestingly replicated at the cellular level, where haemodynamic fMRI change in the putamen can be shown to have a similar property with differing time intervals (Hinton and Meck, 1997). These observations have informed efforts to create a model for temporal processing.

The pacemaker-accumulator model: This is traditionally used to explain how the scalar property occurs in both behavioural and cellular analyses of interval timing (Gibbon, 1977, Gibbon and Allan, 1984, Gibbon *et al.*, 1984, Treisman, 1963). The components are a

pacemaker producing regular pulses, collected by an accumulator. This data, stored in working memory, can be compared to stored reference durations (obtained from prior repeated exposures to stimuli) to estimate duration. The explanation here for the scalar property is the assumption that the error rate during accumulation of the new stimulus will be proportion to its duration (i.e. accumulation of pulses in measuring short durations will be more accurate than longer ones). The pacemaker is presumed to be dopaminergic and the stored reference values for comparison dependent on acetylcholine. Dopaminergic blockage interferes with the pacemaker; for example haloperidol acutely slows the accumulation of pulses (with acclimatisation and recovery of function over repeated doses) (Meck, 1996), and the degree of slowing with dopaminergic blockers is convincingly correlated with D2 affinity of the agents (Meck, 1986). Similar properties can be demonstrated using acetylcholine-modulating drugs (Meck, 1996). However, this simplistic model of autonomy and independence of dopaminergic system in the measurement of time is challenged in behavioural experiments; for example the estimated durations of 2 intervals in Parkinson's patients off medication tended to drift towards each other (rather than predictably shift) in one study (Malapani *et al.*, 1998).

Prospective vs. Retrospective Temporal Processing: It is commonly held that the pacemaker-accumulator model is the basis for ongoing prospective evaluation of time intervals. However, the retrospective evaluation of a previously experienced time interval draws on contextual episodic memory, thought to be accumulated in an automatic manner by the dominant prefrontal cortex, relayed through the hippocampus and retrieved by the non-dominant prefrontal cortex when required (Fuster, 2000, Tulving *et al.*, 1994, Zakay and Block, 2004). In situations where attention cannot be adequately applied to prospective

time evaluation, interval evaluation ability approaches that of the retrospective model (Zakay and Block, 2004).

STRUCTURES INVOLVED IN TEMPORAL PROCESSING

Lessons from animal models implicate a number of structures in time perception. The Macaque (Rhesus) monkey can be trained to model time perception, and other sensory phenomena, through a performance-reward system. In a study by Leon and Shadlen, the animals were trained to evaluate whether a light was illuminated for longer or shorter than a trained standard, and indicate their choice via a saccadic eye movement to one of two targets. Neural activity was measured in the equivalent of the ***posterior parietal cortex*** in regions with neurons that fired only with eye movement to one or other target. It was demonstrated that specific neural activity occurred within 100msec of appearance of the target stimuli and persisted until eye movement occurred. There was an initial liability to activation of the neurons towards the "shorter" target (in itself an interesting finding of non-random preference), but this evolved over the course of the experiment to favour the "longer" target direction. While this direct neuronal measurement was less accurate (~70%) than the monkey's actual choice (~90%), the conclusion was that the posterior parietal region was involved in the monitoring of time/duration perception in addition to more established roles of spatial attention and decision making . The authors argue that it is not merely a stage in motor planning as results did not correlate with actual outcome, nor were the findings explained by attention for the same reason (Leon and Shadlen, 2003). A further similar study examined the same region using a paradigm requiring rhesus monkeys to change fixation to another point as soon as the first target dimmed. The latency was drawn

from either a unimodal or bimodal distribution. Again, neuronal activity in neurons from the lateral intra-parietal area (equivalent of the posterior parietal cortex) demonstrated modulation of resting firing rate related to the expected latency of the next stimulus, based on the pattern experienced by the monkey in each block. This again supported the idea that duration is monitored or encoded in this region (Janssen and Shadlen, 2005). The ***prefontal cortex***, a region typically associated with goal selection and short term memory, has also been associated time perception in further study of Rhesus monkeys. In this case there were 3 targets to indicate whether a central icon was displayed for 1, 1.5 or 2 seconds. The cell recording findings in the prefrontal cortex demonstrated transient phasic modulation of neuronal activity depending on the duration of the previously displayed stimulus that was not correlated with reaction time. Due to the small variations seen with different intervals, the authors conclude that the region is involved with practical indexing of current time intervals relevant to on-going tasks, rather than precise measurement (Genovesio *et al.*, 2006). The importance of **dopaminergic pathways**, as outlined in the previous section, naturally implies the role of the basal ganglia in temporal processing, and the involvement of the **thalamo-cortico-striatal circuits** in time processing is supported by electrophysiological studies (Lewis *et al.*, 2003, Macar *et al.*, 2002, Nieder and Miller, 2003). This circuit primarily involves the supplementary motor area, caudate-putamen, pallidum and thalamus, modulated by dopamine, (Harrington *et al.*, 2004) and also draws on the prefrontal cortex and the posterior parietal cortex; and there is the suggestion that the parietal component may evaluate not only duration, but order and sequence as well as magnitude (Coull *et al.*, 2000, Nieder *et al.*, 2002, Nieder and Miller, 2004, Sawamura *et al.*, 2002). In the striatum, the rate of firing of neurons can be shown to change during temporal processing tasks, and in one interesting study, distinct populations of neurons were

activated with different durations (10s vs 40s) (Matell *et al.*, 2003). This importantly differentiates the processing of these neurons from simple motor function and is supported by other work that implies that continual firing of striatal neurons during prospective evaluation of a time interval is an important component of the process (Apicella *et al.*, 1992, Fiorillo *et al.*, 2003, Schultz *et al.*, 1992). Interestingly, is appears that the striatum is even able to monitor multiple durations in parallel, but only with an intact prefrontal cortex, presumably required to facilitate allocation of attention (Coull and Nobre, 1998, Olton *et al.*, 1988, Pang *et al.*, 2001). Functional MRI studies have consistently associated the basal ganglia, in particular the putamen, with temporal processing . Further work has demonstrated the relevance of the ***pre-supplementary and supplementary motor areas i***n time processing. Mita et al examined these regions in rhesus monkeys in an experiment that required the animals to continue pressing a button for 2, 4 or 8 seconds depending on a visual cue. They found that individual neuron activity was for the most part selective for one of the three time intervals (suggesting neurons in these regions are involved in using time intervals to help plan movement), and that there was build-up or decay of activity over time (suggesting a role in accumulation of data in elapsed time) (Mita *et al.*, 2009). The ***insula*** is also now recognised to play a role in timing. Early suggestion that this may be the case with an insular lesion (Griffiths *et al.*, 1997) has been borne out by fMRI studies that show unilateral (Rao *et al.*, 2001) or bilateral (Jantzen *et al.*, 2004, Jantzen *et al.*, 2005, Livesey *et al.*, 2007, Stevens *et al.*, 2007) insular involvement in a variety of temporal processing tasks. While most studies indicate the anterior insula is involved in short and long time interval processing, the posterior insula may also have a role to play in accumulating time date (Wittmann *et al.*, 2010). There is a suggestion that task difficulty has an effect on the amount of insular activation, with more difficult tasks resulting in greater activation (Deary

et al., 2004, Tregellas *et al.*, 2006) and some hypothesize that the insula has a role in the later stages of temporal processing, at the decision-making stage (Harrington *et al.*, 2004, Livesey *et al.*, 2007). An extension of this is the concept that the insula is involved in all tasks that require comparison of accumulating data to prior references, as evidenced by studies showing similar activation in both temporal and other discriminatory tasks (Ferrandez *et al.*, 2003, Nenadic *et al.*, 2003). In addition to the above, there is significant evidence for prominent involvement of the cerebellum (Ivry, 1997, Ivry and Spencer, 2004, Ivry *et al.*, 2002).

One of the problems in examining the structures involved in temporal processing is the widespread activation that occurs. Not all of these regions are primarily involved in temporal processing but may relate to required associated functions such as memory and attention. This is also affected by the type of task, magnitude of duration (less than one second, seconds or longer) and the predictability of the task. In this way, disruption to some or all of these structures may impair temporal processing, depending on the task used and the method of measurement. For example, in tapping tasks that require repetition of relatively short durations, the regions that are consistently involved are the supplementary motor area, the primary motor cortex and the primary somatosensory cortex. In tasks that involve longer durations and invoke less motor planning and execution, the dorsolateral prefrontal cortex, intraparietal sulcus and premotor cortex seem to be more relevant. Overall, the supplementary motor area, basal ganglia and cerebellum are persistently active in all of these tasks and teasing out their relative contributions may require more intricate task-control subtractions, if possible (Lewis and Miall, 2003).

MODELS OF TEMPORAL PROCESSING

There is evidence for a more complex system than the pacemaker-accumulator model outlined above. An extension of this model is the attentional-gate model, in which temporal processing requires allocation of resources not only to evolution of time but also to non-temporal processes, for example attention and memory (Block and Zakay, 1997). The story does not end there, however. In Parkinson's Disease patients off treatment, the scalar property is lost (Malapani *et al.*, 1998), and can be re-instated by dopamine replacement but equally by stimulation of the sub-thalamic nucleus, indicating a pure dopaminergic system is not involved. Huntington's Disease allows further localisation of function; peri-symptomatic patients (near predicted age of onset) show poor interval timing, implicating involvement of the medium spiny neurons that degenerate in this condition. An fMRI study in patients near and far from predicted age at onset showed hypoactivation in the thalamus, caudate, anterior cingulate and pre-supplementary motor area during an interval discrimination task and implied compensatory hyperactivation in the anterior cingulate and pre-supplementary motor area in patients far from disease onset (Paulsen *et al.*, 2004). In patients with cerebellar lesions, the scalar property is preserved. It has been proposed therefore that different circuits may exist for recording of episodic intervals involving the cerebellum (in the millisecond range), and for continuous recording (in the seconds range) involving the basal ganglia and associated structures (and dependent on attention) (Ivry and Spencer, 2004, Meck, 2005, Pfeuty *et al.*, 2003). Further evidence for divergent roles of the cerebellum and basal ganglia systems in temporal processing comes from studies of motor performance (in which tasks involving only attention were not impaired in cerebellar lesion patients but tasks involving either attention or attention plus motor activity were impaired

in Parkinson's patients) (Ravizza and Ivry, 2001), and studies comparing continuous with discontinuous timing tasks (the latter only being impaired in patients with cerebellar disease) (Spencer *et al.*, 2003). ***Striatal beat frequency*** is an alternative model which incorporates the fact that basal ganglia is not exclusively responsible for temporal processing but rather may monitor thalamo-cortico-striatal loop activity as part of the process. In essence, the theory is that basal ganglia act to monitor neuronal activity in other regions in the thalamo-cortico-striatal circuit; a peak in striatal firing at the onset of a period of duration monitoring synchronises with cortical oscillators and then continual firing reflects attention to the task (possibly spike counting) with a burst of activity at the end of the task reflecting an assessment of duration. This theory is supported by experimental data (Beiser and Houk, 1998, Fries *et al.*, 2001, Galarreta and Hestrin, 2001, Riehle *et al.*, 1997, Salinas and Sejnowski, 2001, Silva *et al.*, 1991, Steinmetz *et al.*, 2000) and seems to demonstrate the scalar property (Matell and Meck, 2004), but fails to address some observations, for example the effect of cholinergic drugs on temporal processing. Further proposed models invoke the notion that memory decay processes are important in temporal processing (Staddon, 2005, Wackermann and Ehm, 2006).

MEASUREMENT OF TDT

The TDTs presented in this paper were measured using pairs of stimuli presented to the subject as follows: Temporal discrimination thresholds (TDTs) are examined in a single session in a sound-proof, air-conditioned room. TDTs are measured for various task types: (1) Visual-visual: two LED lights are used, vertically orientated and placed on the table 60cm in front of the subject. The lights are seven degrees into the subject's peripheral vision on the side of the body being tested. (2) Tactile-tactile: Non-painful, above-threshold electrical

stimulation is used on the second and third fingers on the side of the body being tested using square-wave stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom). Stimulus current is progressively increased from zero in 0.1mA steps to the lowest point at which the subject can reliably detect the impulse (tested using a paradigm with 10 trials of randomly assigned real or sham impulses requiring a response from the subject). Equality of stimulus intensity is then established between the digits if necessary. The stimulus current required typically ranges between 2mA and 4.5mA. (3) Visual-Tactile: A combination of one LED light and stimulation of one finger on the same side is used with the same equipment.

The specific instructions provided to participants during the TDT session are as follows;

We will be performing the test in three different ways – using two lights (the 'visual' task), using two fingers (the 'tactile' task) or using a mixture of one light and one finger (the 'mixed' task). I will tell you before each run which type of test is being performed. For all types of task you need to focus on the central fixation point throughout the task.

The visual test uses the two flashing lights on the table, which you will detect in your peripheral vision. It is important that you look at the central red fixation point at all times and not at either light. The lights flash together as a pair every 5 seconds. At the start they are at exactly the same time – perfectly synchronized. At some point the lights start to become separated in time so that one light flashes slightly before the other. As time passes, the lights may get further apart.

For each pair of flashes I need a one-word response. The responses are:

"Same" – the lights are perfectly synchronized – at the same time - as they will be at the start.

"Different" – the lights are not at exactly the same time: you are relatively certain that they are not synchronized but you aren't able to tell which is first.

"Top" or "Bottom" – the top or bottom light definitely illuminates earlier.

The tactile task small electrical impulses to the stickers on you index and middle finger on one hand. You must look at the red fixation point at all times. Similar to the lights, you feel the small impulse or tap on both fingers at exactly the same initially – perfectly synchronized - and then at some point they start to become separated in time. As time passes, the impulses may get further apart.

For each pair of finger taps I again need a one-word response. The responses are:

"Same" – the finger impulses are perfectly synchronized – at the same time – as they will be at the start.

"Different" – the finger taps are not at exactly the same time: you are relatively certain that they are not synchronized but you aren't able to tell which is first.

"Index" or "Middle" – the impulse to the index or middle finger is definitely earlier.

Finally the mixed task involved stimulation using one light with an impulse to one finger. Again, you must look at the red fixation point at all times. The top light flashes at the same as the middle finger on the same side receives an impulse. Nothing happens with the bottom light or the index finger – there are only two stimuli as before. At the start the light and finger impulse occur at the same time – perfectly synchronized. At some point the stimuli start to become separated in time and may get further apart with time.

For each pair I need a one-word response. The responses are:

"Same" – the light and finger tap are at exactly the same time, as will be are at the start.

"Different" – the stimuli are not at exactly the same time: you are relatively certain that

they are not synchronized but you aren't able to tell which is first.

"Light" or "Finger" – the Light or finger stimulus is definitely earlier.

Each of the tasks is performed four times on each side of the body in random order, resulting in a total of 16 runs where 2 tasks (visual and tactile only) are used and 24 runs (where all three task types are used) per subject. Task order is randomized to minimize practice or attention effect. Pairs of stimuli are synchronized initially and were progressively separated in 5ms steps. When the subject reports that the pairs of stimuli are asynchronous on three consecutive occasions, the first of these is taken as the TDT. The median of the four runs for each condition (tasks x sides) is used for each subject to allow for practice effect and these results are averaged to obtain a summary (combined) TDT score. Results of the combined TDT are shown with their standard deviation (SD) and 95% confidence intervals (CI). The TDT results presented in this thesis are:

- ***2-task TDT (visual and tactile)*** in Chapter 2 (later TDT results), Chapter 5 (section on phenotypes), Chapter 6, and Chapter 7.
- ***3-task TDT (visual, tactile and mixed)*** in Chapter 2 (initial TDT results), Chapter 3, Chapter 4, and Chapter 5 (section on task comparisons).

STANDARDISED SCORES

The combined TDT score (the average of the results for the two or three task types) is used in analyses to assign status to subjects. Using the mean and standard deviation of the TDTs of the control group, standardized Z-scores are calculated for all subjects using the formula;

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

Z- scores of equal to or greater than 2.5 are considered abnormal.

OTHER CANDIDATE ENDOPHENOTYPES

In addition to TDT, a number of other candidate endophenotypes have been investigated in AOPTD including the spatial discrimination threshold (see below), vibration-induced illusion of movement (VIIM) (Rome and Grunewald, 1999), Positron Emission Tomography (PET) (Eidelberg *et al.*, 1998), Diffusion Tensor Imaging (DTI),(Carbon *et al.*, 2004) and transcranial magnetic stimulation (TMS) (Edwards *et al.*, 2003). These will be considered in more detail in Chapter 4.

THE SPATIAL DISCRIMINATION THRESHOLD

The spatial discrimination threshold is a measure of spatial acuity taken at the index finger. This relatively easy to administer sensory test has been examined at the Department of Neurology at St. Vincent's University Hospital (O'Dwyer *et al.*, 2005, Walsh and Hutchinson, 2007, Walsh *et al.*, 2007, Walsh *et al.*, 2009) and a comparison between SDT and TDT forms part of this thesis.

The spatial discrimination threshold (SDT) is a measure of spatial acuity and is determined using a grating orientation task employing Johnson-van Boven-Philips (JVP) domes applied to the fingertip. Abnormal SDTs have been found in AOPTD patients as well as their unaffected relatives (Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Sanger *et al.*, 2001, Walsh *et al.*, 2007). In addition to disordered sensory processing in the basal ganglia, abnormal representation in the primary sensory cortex (S1) may be important in the causation of SDT abnormalities (Bara-Jimenez *et al.*, 1998, Meunier *et al.*, 2001). Plasticity in S1 may explain some of the variability of SDT results, including improvement with botulinum toxin treatment (Walsh and Hutchinson, 2007).

MEASUREMENT OF SDT

Spatial Discrimination Thresholds are measured at the index fingertip using Johnson-van Boven-Philips domes (a test of spatial acuity using grating orientation). It is known that the greatest spatial acuity, and therefore the most sensitive areas to examine for abnormal SDTs, are the fingertips and lips (Van Boven and Johnson, 1994). The experimental technique for measurement in SDT in the Department of Neurology has evolved since 2003 (O'Dwyer *et al.*, 2005) and is summarized here:

The Johnson-van Boven-Phillips (JVP) Domes used in measuring SDT are hemispheric plastic domes with gratings of various widths (0.35mm to 3mm standard set, up to 4.5mm available) and are commercially available (Stoelting Co., Illinois). The well documented ceiling effect when testing SDT above age 65 (due to age-related deterioration in the peripheral nervous system) limits the usefulness of the test over this age.

Spatial discrimination thresholds are measured determined at the skin overlying the distal fat pad of the index finger on each side. The test is carried out at a table with the participant seated opposite the examiner at a suitable height. The participant is separated from the examiner using an opaque screen with the index finger being tested extended under the screen and accessible by the examiner. The test is started using the largest grating width available (in our Department 4.5mm) and the dome is applied to the index finger pad either horizontally (parallel to long axis of finger, **"down"**) or vertically (perpendicular to long axis of finger, **"across"**) 20 times for 1-2 seconds. The order is randomized and subjects were asked to respond immediately with their impression of the orientation, and to guess if not sure. No feedback is provided. The angle of application is kept constant to remove alternative sensory information that could inform the correct answer. Serially smaller grating-width domes are used (each applied 20 times) until less than 60% of answers (12 of 20 applications) are correct. The SDT for each is calculated (by linear interpolation of the 75% level of accuracy) using the formula:

$$STD = \frac{W^- + (W^+ - W^+) * (0.75 - P^-)}{(P^+ - P^-)}$$

W^+ = the largest width that scored less than 75% correct

W^- = the smallest width that achieved greater than 75% correct

P^- and P^+ are the fraction of correct responses at W^- and W^+

Subjects that could not exceed 15/20 correct responses for the initial 4.5mm grating are assigned a results of 4.5mm for that hand. The overall SDT result is the average of the results from both sides.

THESIS OBJECTIVES

Following a review of the literature, the following research questions remain and form the objectives of this thesis;

1. To examine TDT in healthy control subjects.
2. To examine the profile of TDT results amongst sporadic (no other family member affected) and familial (positive family history) patients with adult onset primary torsion dystonia (AOPTD) as validated by a standardized neurological assessment.
3. To examine TDT in the unaffected relatives of both sporadic and familial AOPTD patients.
4. To investigate the pattern of TDT inheritance in familial AOPTD pedigrees and in sporadic cervical dystonia families.
5. To correlate TDT and structural imaging findings (using voxel-based morphology) in AOPTD patients, unaffected relatives and healthy controls.
6. To investigate what differences can be demonstrated between AOPTD patients, unaffected relatives and healthy controls using functional MRI.

To examine how temporal discrimination thresholds compare to spatial discrimination thresholds, a sensory test previously examined as a candidate endophenotype in the Department of Neurology at St. Vincent's University Hospital, and other published candidate endophenotypes in dystonia.

CHAPTER 2
TEMPORAL DISCRIMINATION THRESHOLD FINDINGS IN CONTROL SUBJECTS, AOPTD PATIENTS AND UNAFFECTED RELATIVES

Following on from the discussion in Chapter 1, and the difficulties encountered during genetic studies of adult-onset primary torsion dystonia (AOPTD) to date, this Chapter focuses on an investigation of temporal discrimination threshold (TDT) testing (a relatively simple examination of sensory discrimination ability) in AOPTD patients and their unaffected relatives, with a view to establishing whether it conforms to the characteristics of a sensitive endophenotype capable of assisting in future genetic study of the condition.

Adult-onset primary torsion dystonia (AOPTD) is the most common form of dystonia; most patients appear to have sporadic AOPTD but up to 25% of these have another affected family member (Leube *et al.*, 1997, Stojanovic *et al.*, 1995). Familial AOPTD is inherited in an autosomal dominant fashion with a penetrance as low as 12-15% (Waddy *et al.*, 1991); the paucity of multiplex AOPTD families makes genetic study of the disorder difficult. The use of a sensitive endophenotype, a marker of subclinical gene carriage in unaffected relatives, is one approach to this problem.

Significant sensory processing abnormalities are found in AOPTD patients including abnormalities in spatial discrimination threshold (SDT), temporal discrimination threshold (TDT) and vibration induced illusion of movement (VIIM) (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et*

al., 2005, Walsh *et al.*, 2007). These sensory abnormalities may be of utility as endophenotypes. In addition, it has been proposed that abnormal sensory processing may be an important primary phenomenon in AOPTD, and may play a role in the pathogenesis of AOPTD (Hallett, 1995, Tinazzi *et al.*, 2003).

Spatial discrimination thresholds (SDTs) are abnormal in some unaffected relatives of AOPTD patients (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007) and have been investigated as an endophenotype. However the prevalence of abnormal SDTs in AOPTD patients is low and a more sensitive marker of gene carriage is needed which might significantly aid genetic research.

The Temporal Discrimination Threshold (TDT) is the shortest time interval at which a subject can detect that two stimuli are asynchronous; TDT testing is psychophysiological task that is relatively easy to administer with the advantage of showing significantly less age-dependence than other candidate sensory tests in AOPTD such as spatial discrimination thresholds (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). One study by Hoshiyama and colleagues, for example, showed little effect of age on TDT up to 65 years (Hoshiyama *et al.*, 2004). The TDT has been shown to be abnormal in DYT1 patients and non-manifesting DYT1 carriers compared to non-carrier relatives or controls (Fiorio *et al.*, 2007). The TDT has also been shown to be abnormal in patients with writer's cramp (Fiorio *et al.*, 2003), blepharospasm (Fiorio *et al.*, 2008), Parkinson's disease (Artieda *et al.*, 1992, Lee *et al.*, 2005) and multiple system atrophy (Lyoo *et al.*, 2007) and therefore may be a sensitive marker of abnormal sensory integration in the basal ganglia. An early study of temporal discrimination in subjects with focal cerebral lesions found that TDT was increased without evident sensory loss in lesions involving the putamen (Lacruz *et al.*, 1991). fMRI studies of

both spatial and temporal discrimination tasks evoked basal ganglia activation (Pastor *et al.*, 2004), and during an auditory temporal discrimination task activation in the basal ganglia occurred early and was uniquely associated with encoding time intervals (Rao *et al.*, 2001). Pastor and colleagues suggested that disorders affecting the basal ganglia would affect both spatial and temporal discrimination (Pastor *et al.*, 2004).

These studies all suggest that TDT may be functioning as an endophenotype in AOPTD by identifying subclinical basal ganglia dysfunction; however this has not been investigated by examining both AOPTD patients and their unaffected relatives. The finding that TDT abnormalities act as a marker of non-penetrant gene carriage in unaffected relatives would be useful in performing genetic studies of the disorder.

The aim of this study was to investigate the potential use of TDT as an endophenotype by measuring the prevalence of TDT abnormalities in familial and sporadic AOPTD patients, their unaffected relatives and healthy control subjects. It was hypothesized that an abnormal TDT in clinically unaffected relatives of AOPTD patients is a marker of subclinical gene carriage, and that appropriate frequencies of abnormal TDTs would be observed in patients and relatives. It was further hypothesized that in multiplex AOPTD families the transmission of TDT would be consistent with an autosomal dominant endophenotype. This Chapter reports the results of my initial TDT study, and the figures are revisited at the end of the Chapter with additional subjects recruited over the period of the research fellowship.

PATIENTS AND METHODS

AOPTD PATIENTS

35 AOPTD patients (17 familial, 18 sporadic) (mean age 53; range 35 to 73) with focal dystonia (20 cervical dystonia, 13 focal hand dystonia, one spasmodic dysphonia, one musician's dystonia) were recruited from the cohort at St. Vincent's University Hospital. The clinical diagnosis of these patients was assessed using a videotaped neurological examination reviewed by two neurologists with expertise in dystonia. The majority of the familial patients came from six multiplex families; the index cases of these families were DYT1 negative. The remaining patients did not have routine DYT1 screening in keeping with guidelines (Albanese *et al.*, 2006, Bressman *et al.*, 2000) as all had onset after the age of 26 years with no family history of early-onset dystonia. Eighteen of the 35 patients were receiving regular botulinum toxin injections for their dystonia. The mean (standard deviation) time since last injection in these 18 individuals was 8.2 (14.2) weeks.

UNAFFECTED RELATIVES

Forty-two first-degree relatives (26 of familial cases, 16 of sporadic cases) and 32 second-degree relatives (all of familial cases) were recruited (mean age 42 years; range 19 to 76). All were examined clinically using a protocol for evidence of dystonia; none had any evidence of dystonia or dystonic tremor.

CONTROL PARTICIPANTS

From hospital staff and visitors to the hospital, 43 healthy control subjects were recruited. These were divided into two groups; under 50 years of age (n=26; mean age 31 years; range 22-49) and over 50 years (n=17; mean age 58 years, range=50-71). Exclusion criteria were a history of neurological disease including neuropathy, visual disorder or a history of cerebral, cervical or brachial plexus injury.

All subjects had normal cognition, normal visual acuity, absence of sensory symptoms and a normal sensory examination.

SENSORY TESTING

Temporal discrimination thresholds (TDTs) were examined in a single session in a sound-proof, air-conditioned room. TDTs were measured for three tasks: (1) Visual-visual: two LED lights were used, horizontally orientated and placed on the table in front of the subject. The lights were seven degrees into the subject's peripheral vision on the side of the body being tested. (2) Tactile-tactile: Non-painful, above-threshold electrical stimulation was used on the second and third fingers on the side of the body being tested using square-wave stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom). Stimulus current was progressively increased from zero in 0.1mA steps to the lowest point at which the subject could reliably detect the impulse (tested using a paradigm with 10 trials of randomly assigned real or sham impulses requiring a response from the subject). Equality of stimulus intensity was then established between the digits if necessary. The stimulus current required ranged between 2mA and 4.5mA. (3) Visual-Tactile: A combination of one LED light

and stimulation of one finger on the same side was used with the same equipment. Each of the three tasks was performed four times on each side of the body in random order, resulting in a total of 24 runs per subject. Task order was randomised to minimise practice or attention effect. Pairs of stimuli were synchronised initially and were progressively separated in 5ms steps. When the subject reported that the pairs of stimuli were asynchronous on three consecutive occasions, the first of these was taken as the TDT. The median of the four runs for each condition (3 tasks x 2 sides) was used for each subject to allow for practice effect and these six results were averaged to obtain a summary (combined) TDT score. Results of the combined TDT are shown with their standard deviation (SD) and 95% confidence intervals (CI).

Analysis: The combined TDT score (the average of the results for the three task types) was used in analyses to assign status to subjects; side of body and task type were also analysed as within-subject factors. Unless otherwise stated, TDT refers combined TDT in the results and discussion. All statistical analyses of behavioural data were conducted using Minitab 15. Groups (AOPTD patients, unaffected relatives, healthy controls) were compared using analysis of variance. Using the mean and standard deviation of the TDTs of the control group, standardised Z-scores were calculated for all subjects using the formula;

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

Z- scores of equal to or greater than 2.5 were considered abnormal.

RESULTS

CONTROL SUBJECTS

There was a statistically significant effect of age on the combined TDT score when the control group was considered as a whole; a linear regression revealed a statistically significant correlation between raw TDT result (in milliseconds) and age ($p<0.0001$) (Figure 2.1). Control subjects were divided into two groups under 50 years (n=26; mean 31 years; range 22-49) and over 50 years (n=17; mean 58 years, range 50-71) to allow age-related normal values to be calculated; within each of these two groups a linear regression did not reveal a statistically significant correlation between TDT and age ($p=0.18$ for the under 50yo group and $p=0.08$ in the over 50yo group) (Figures 2.2 and 2.3). The mean TDT in the under 50 control group was 22.85 ms (SD 8.00: 95% CI; 19.62-26.09 ms). The mean TDT in the over 50 control group was 30.87 ms (SD 5.48: 95% CI; 28.05-33.69 ms). The upper limit of normal, defined as control mean + 2.5 SD, was 42.86 ms in the under 50 group and 44.58 ms in the over 50 group. All of the control subjects' Z-scores were less than 2.5 (range -2.21 to +1.76).

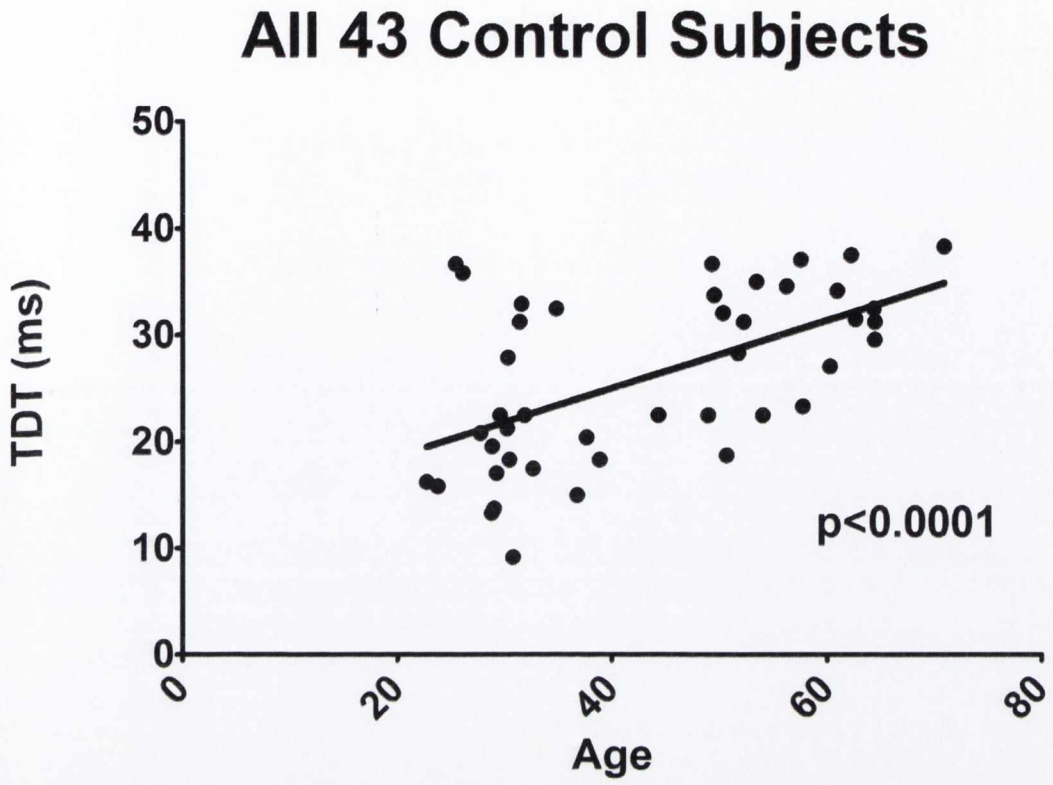


Fig 2.1: *Scatterplot of TDT (ms) against age in all 43 control subjects, with linear regression (line) associated with a statistically significant correlation between TDT (ms) and age (years) (p<0.0001).*

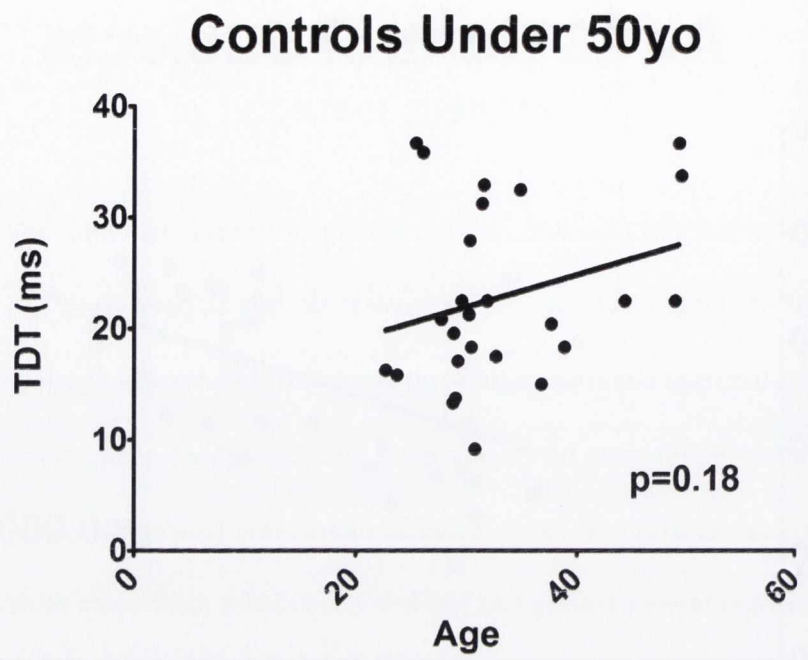


Fig 2.2: *Scatterplot of TDT (ms) against age in 26 control subjects under 50 years, with linear regression (line) showing no statistically significant correlation between TDT (ms) and age (years) (p=0.18).*

Fig 2.3: *Scatterplot of TDT (ms) against age in17 control subjects over 50 years, with linear regression (line) showing no statistically significant correlation between TDT (ms) and age (years) (p=0.08).*

AOPTD PATIENTS

30/35 (86%) AOPTD patients had abnormal TDTs compared to controls; the frequency of abnormalities was similar in sporadic (16/18; 89%) and familial (14/17; 82%) patients (Fisher's Exact test; p=0.658) (Figure 2.4; Table 2.1). There was also a similar frequency of abnormalities when comparing cervical dystonia (19/20; 95%) and focal hand dystonia (10/13; 77%) patients (Fisher's Exact Test; p=0.276) (Figure 2.5(b)). In the 18 patients treated with botulinum toxin there was no statistical correlation between TDT and time since last botulinum toxin injection.

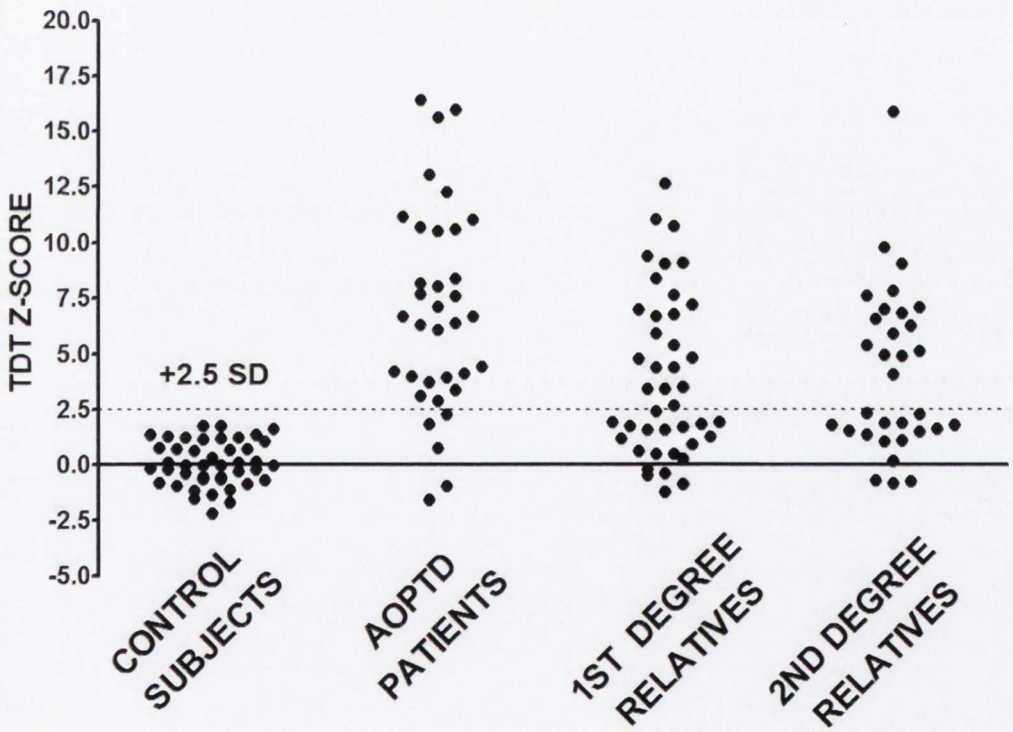


Figure 2.4: *Z-scores for Temporal Discrimination Threshold (TDT). The Z-scores of 43 healthy control subjects ranging from -2.21 to +1.76 are illustrated in the column on the left. 30 of 35 (86%) AOPTD patients (17 familial; 18 sporadic), 22 of 42 (52%) first degree relatives (26 familial; 16 sporadic) and 16 of 32 second degree relatives (all familial) had abnormal TDTs using a cut-off of 2.5 standard deviations (Z-SCORE = 2.5) above the control mean (dotted line). (1ST DEGREE RELATIVES = Unaffected first degree relatives of AOPTD patients; 2ND DEGREE RELATIVES = Unaffected second degree relatives of AOPTD patients.)*

UNAFFECTED RELATIVES

The frequency of TDT abnormalities amongst the first degree relatives was 52% (22/42) (Figure 2.4; Table 2.1); the frequencies in familial relatives (15/26; 57%) and sporadic relatives (7/16; 44%) were similar (Fisher's Exact Test; p=0.527) (Figure 2.5a). Sixteen of 32 second degree relatives had abnormal TDTs. The mean TDT in the patient group was 70.32 ms (SD 26.87; 95% CI 61.09 – 79.55 ms) and in the relatives group was 52.29 ms (SD 24.15; 95% CI 46.69 – 57.88 ms).

The TDTs in AOPTD patients, unaffected relatives and control subjects were statistically significantly different (1-way non-parametric ANOVA p<0.0001; post-hoc comparisons using Tukey 99% simultaneous confidence intervals showed that all 3 groups (patients, relatives and controls) were statistically different from each other). When analysed as a within-subject factor, side of body was non-significant.

	N	Mean TDT	SD	95% CI
Control <50	26	22.85	8.00	19.62 – 26.09
Control >50	17	30.87	5.48	28.05 – 33.69
AOPTD Patients	35	70.32	26.87	61.09 – 79.55
Unaffected Relatives	74	52.29	24.15	46.69 – 57.88

Table 2.1: *Summary of Temporal discrimination Threshold (TDT) testing showing mean, standard deviation (SD) and 95% confidence intervals (95% CI) for each group of control subjects < 50 and > 50 years, adult onset primary torsion dystonia (AOPTD) patients and their unaffected relatives.*

INDIVIDUAL TASKS

The combined TDT results in Figures 2.1-2.4 and Table 2.1 present the mean of the measurements for the three individual tasks (visual, tactile and mixed). When analysed as a within-subject factor in the control group, task type was not significant [$F(2,84) = 2.242$; $p=0.095$]. The combined TDT was chosen to assign TDT status as a mechanism of increasing sensitivity as it uses all of the available temporal discrimination data for each subject. However task type was a significant within-subject factor in the patient [$F(2,64) = 5.460$; $p=0.006$] and relative [$F(2,144) = 18.105$; $p<0.0001$] groups. In keeping with similar studies (Fiorio *et al.*, 2007, Fiorio *et al.*, 2008), the visual task had the lowest TDT followed by the tactile and then the mixed task. Concordance (all 3 individual task results in a particular subject being <2.5 SD "normal" or >2.5SD "abnormal") was not 100%. In using the combined TDT score, some subjects who did not reach the 2.5 SD threshold for abnormality in one task were still assigned abnormal status because the combined result for the three tasks exceeded the cutoff (i.e. some subjects categorised as having an abnormal combined TDT had a Z-score below 2.5 for one of the three tasks).

The three TDT tasks were assessed separately in terms of frequency of abnormalities (Table 2.2). In AOPTD patients, the combined TDT had a sensitivity of 86%. The sensitivity of an abnormal visual TDT was 86%, of an abnormal tactile TDT was 85% and of an abnormal mixed TDT was 67%. Comparing cervical dystonia and writer's cramp patients, the frequencies of abnormalities were similar for the visual task (Fisher's Exact test; $p=0.276$), tactile task (Fisher's Exact test; $p=0.630$) and mixed task (Fisher's Exact test; $p=0.461$) (Figure 2.5(b); Table 2.2). The frequencies of abnormal visual TDT, tactile TDT and mixed TDT in

unaffected first degree relatives were 50%, 45% and 46% respectively; the frequency of abnormalities using the combined TDT was 52%. The concordance [all 3 individual task results in a particular subject being <2.5 SD (normal) or >2.5 SD (abnormal)] in control subjects was 100%. Concordance was lower in AOPTD patients (76%) and unaffected relatives (77%).

	N	VISUAL TDT	TACTILE TDT	MIXED TDT	COMBINED TDT
All Patients	35	86%	85%	67%	86%
Cervical Dystonia	20	95%	89%	75%	95%
Writer's Cramp	13	77%	83%	62%	77%
Spasmodic Dysphonia	1	0/1	0/1	0/1	0/1
Musician's Dystonia	1	1/1	1/1	1/1	1/1

Table 2.2: *Summary of the relative sensitivities of the individual Temporal discrimination Threshold (TDT) tasks and the combination in AOPTD patients with cervical dystonia (n=20), writer's cramp (n=13), spasmodic dysphonia (n=1) and musician's dystonia.*

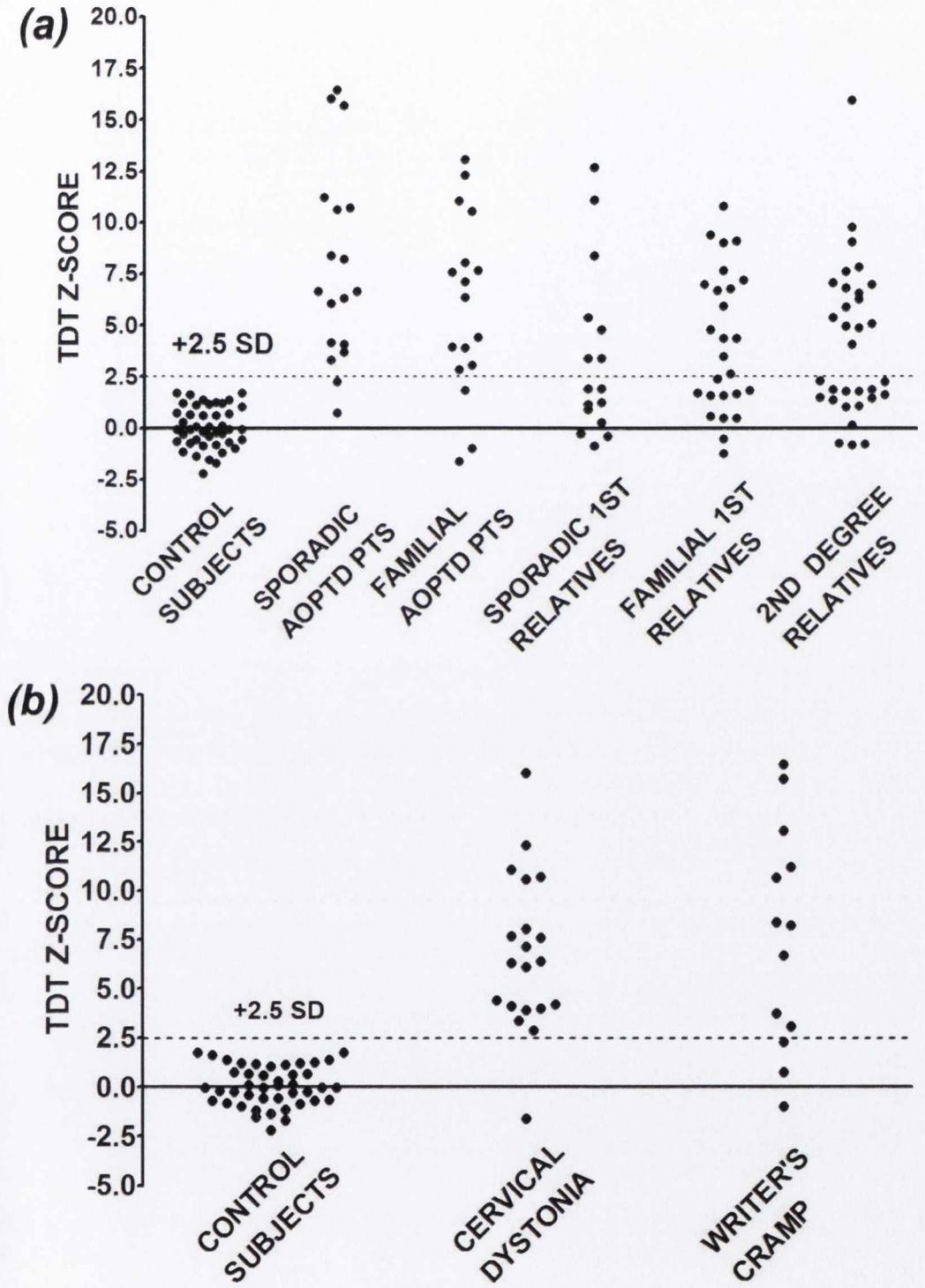


Figure 2.5 (a): *Scatterplot if TDT Z-Scores in control subjects,sporadic and familial AOPTD patients, along with sporadic (all first degree) and familial (first and second degree) relatives;* **(b):** *Scatterplot if TDT Z-Scores in control subjects, 20 cervical dystonia and 13 focal hand dystoina patients; dotted line indicates Z-Score=2.5, with values above this line considered abnormal*

TEMPORAL DISCRIMINATION IN AOPTD FAMILIES

Sixteen of the 20 familial AOPTD subjects tested for TDT came from six multiplex families in which at least three family members were clinically affected; 15 of these 16 had abnormal TDTs. These six families were identified and characterized several years ago by the Department of Neurology (O'Dwyer *et al.*, 2005); as a result of relocation, illness and other factors only a limited number of the previously examined individuals in these pedigrees were available and willing to undergo TDT measurement for the present study. The four remaining familial AOPTD subjects had only one other family member affected. All of the familial unaffected relatives of AOPTD patients (28 first degree and 35 second degree) belonged to the six multiplex families; 15 of 28 unaffected first degree relatives and 19 of 35 second degree relatives had abnormal TDTs. TDT Z-Scores are displayed in Figure 2.6.

The family trees with the TDT Z scores for each family member examined are illustrated in Figure 2.7. Autosomal dominant transmission is demonstrated and it is noteworthy that in pedigree 006 (Fig 2.7) one family member (II:2) was clinically unaffected but was regarded as an obligate carrier due to having an affected child (III:8) and an affected sibling (II:6), this obligate carrier had an abnormal TDT (Z =9.4). Two individuals in pedigree 008 (IV:3 and IV:4) and two in pedigree 006 (II:3 and III:5) who were clinically unaffected with affected siblings were considered obligate endophenotype carriers as some of their clinically unaffected offspring had abnormal TDTs; these obligate endophenotype carriers had abnormal TDTs also.

Using TDT testing in 79 individuals in the six families, 30 had normal TDT Z scores, one of whom had spasmodic dysphonia and 49 abnormal TDT Z scores were identified in 15 affected individuals, one obligate carrier and 33 other unaffected relatives (14 first degree

and 19 second degree). Thus in these six families using TDT as an endophenotype I was able to identify more than twice as many endophenotype carriers as clinically manifesting individuals. No individual who had a normal TDT was found to have an offspring with an abnormal TDT.

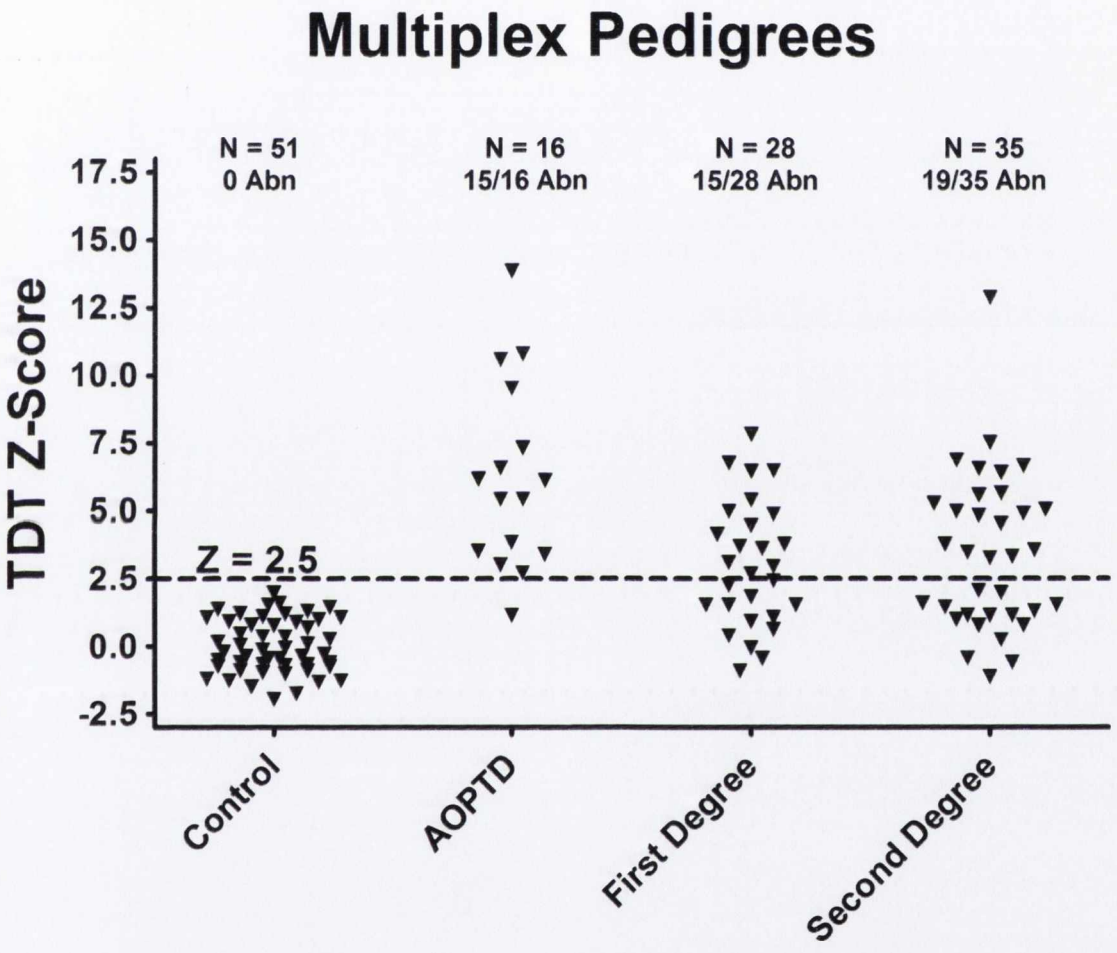
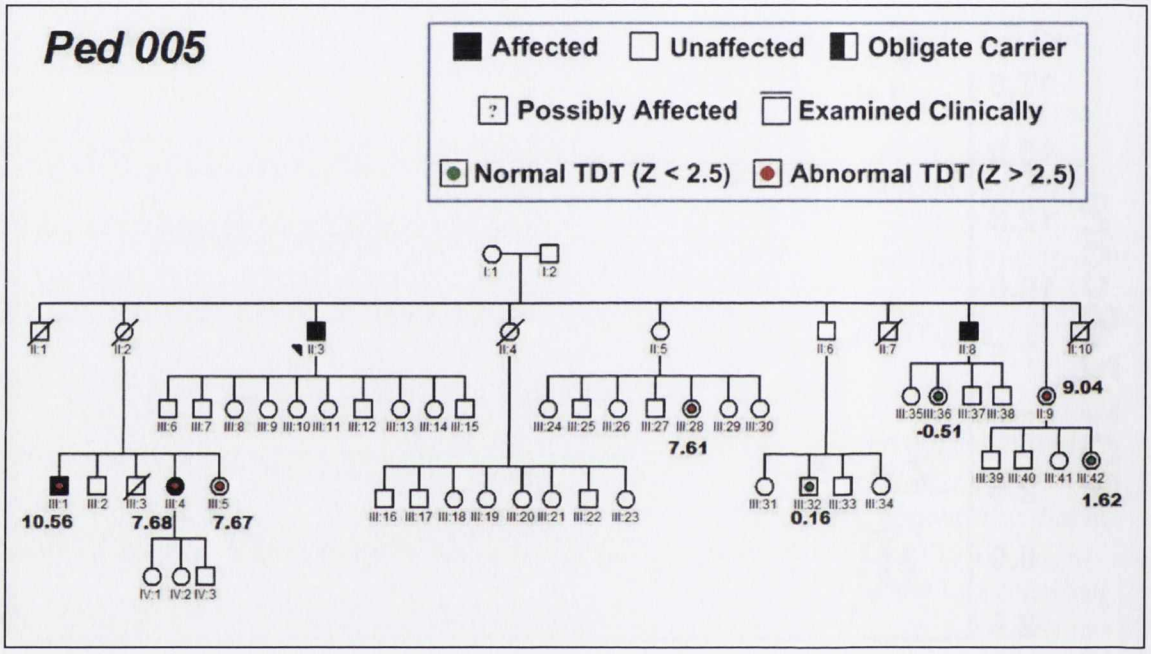
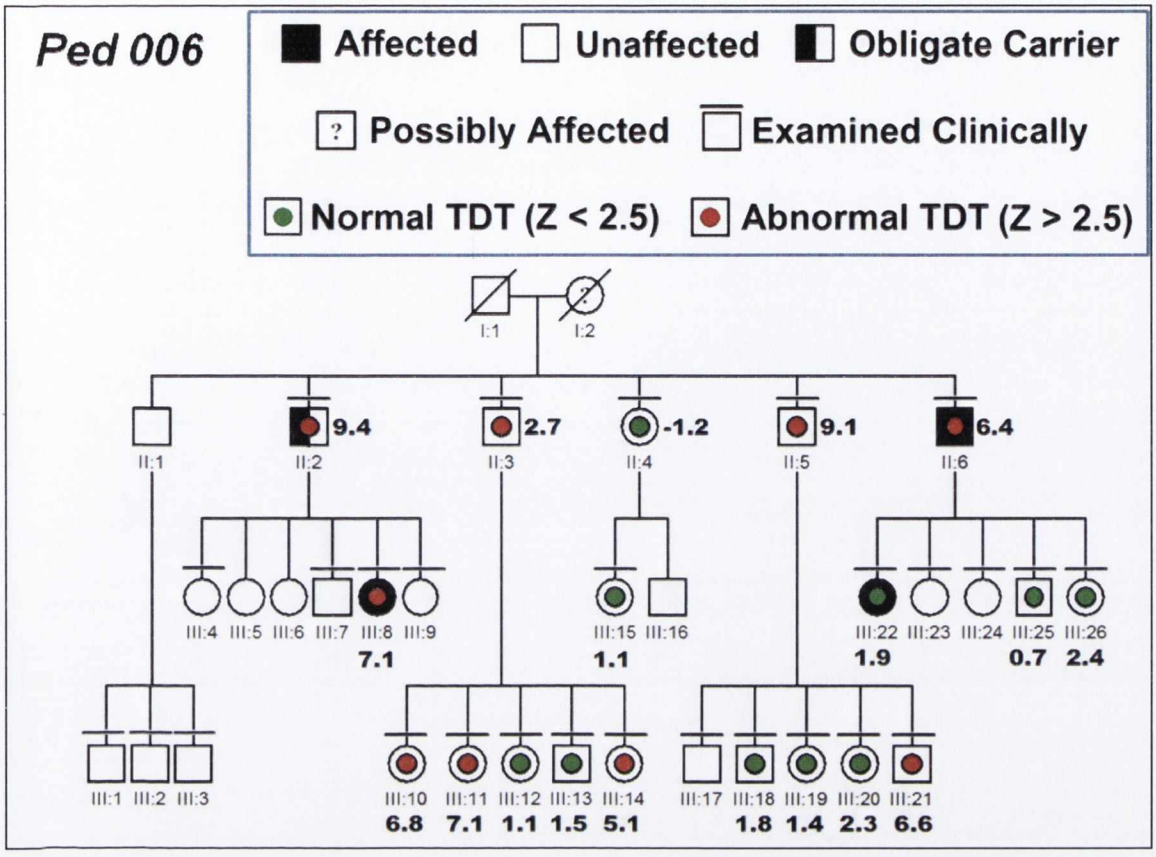
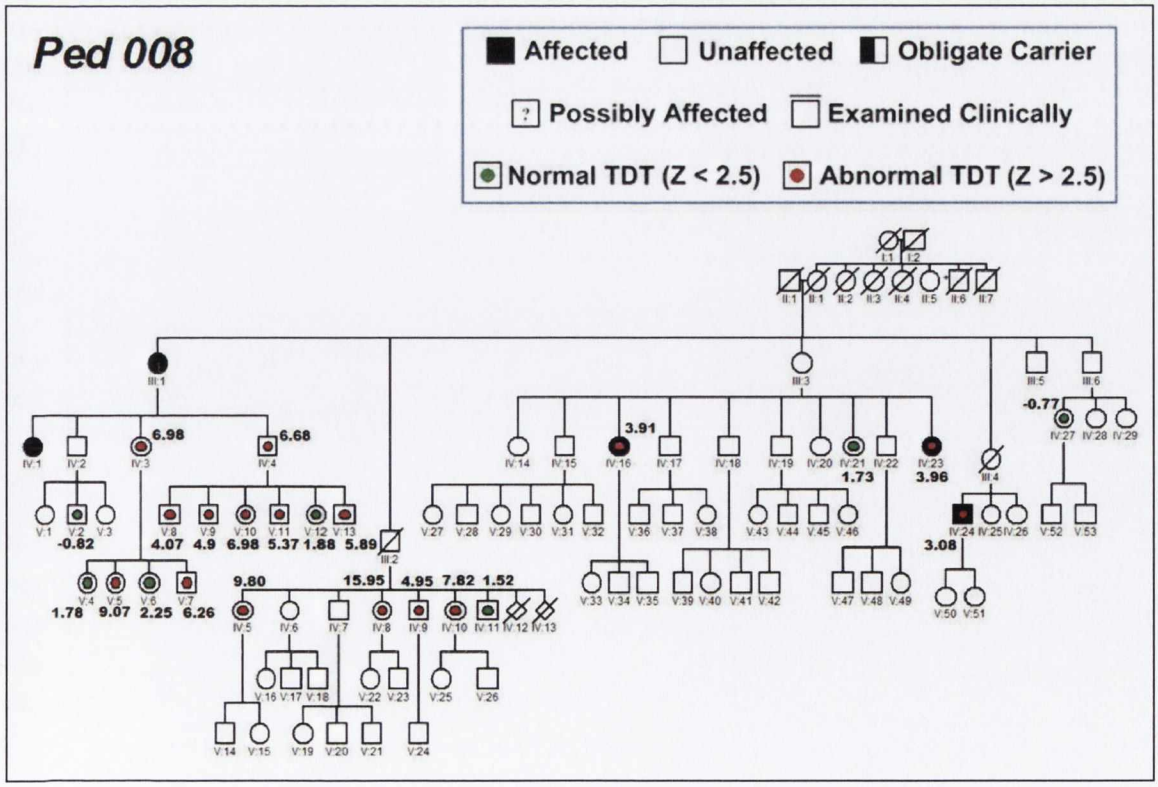
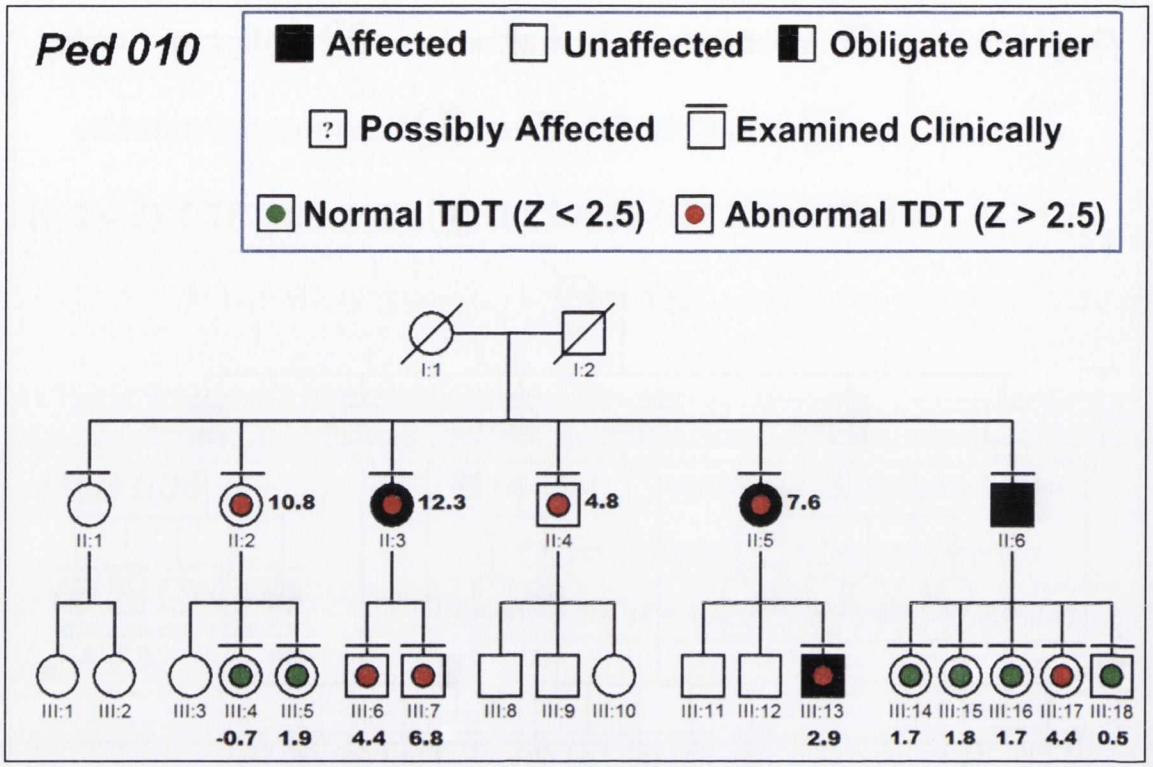


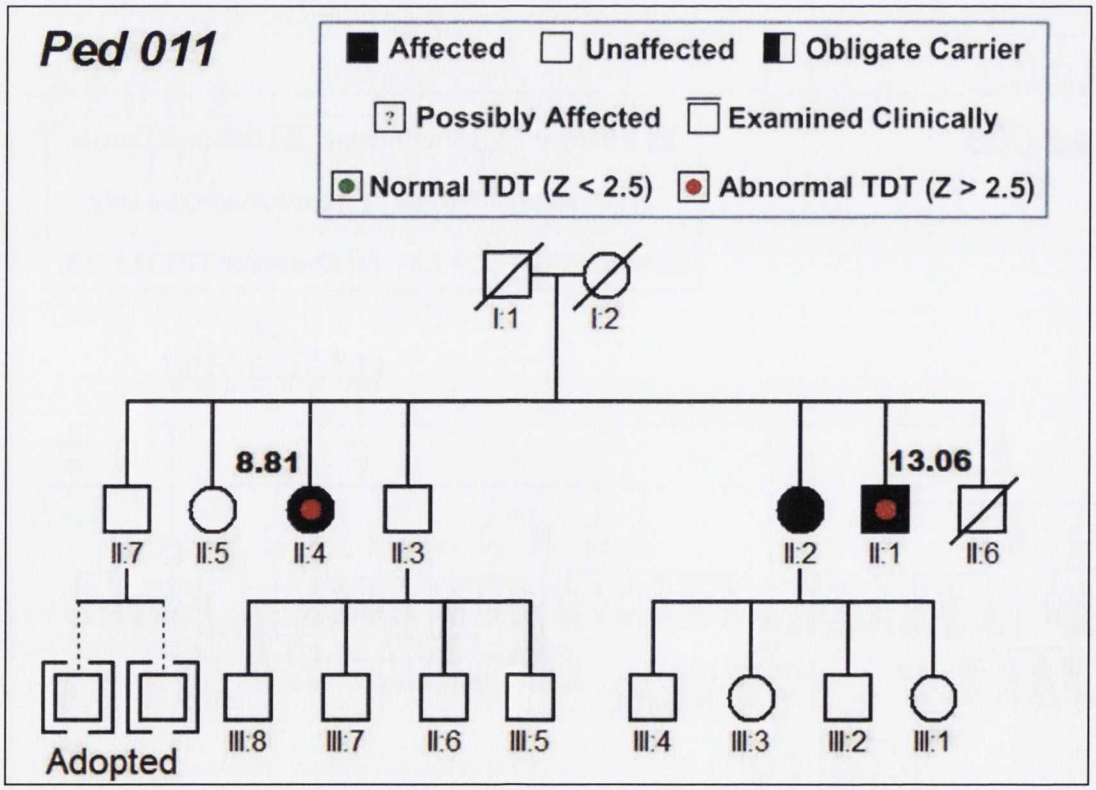
Figure 2.6: *TDT Z-Scores in 43 control subjects and the 16 AOPTD patients and 28 first and 35 second degree unaffected relatives from the 6 multiplex families included in the study.*


Ped 005
Affected
Unaffected
Obligate Carrier
Possibly Affected
Examined Clinically
Normal TDT (Z < 2.5)
Abnormal TDT (Z > 2.5)
I:1 I:2
II:1 II:2 II:3 II:4 II:5 II:6 II:7 II:8 II:10
III:6 III:7 III:8 III:9 III:10 III:11 III:12 III:13 III:14 III:15
III:24 III:25 III:26 III:27 III:28 III:29 III:30
7.61
III:35 III:36 III:37 III:38 II:9
9.04
-0.51
III:39 III:40 III:41 III:42
1.62
III:1 III:2 III:3 III:4 III:5
10.56
7.68
7.67
III:16 III:17 III:18 III:19 III:20 III:21 III:22 III:23
III:31 III:32 III:33 III:34
0.16
IV:1 IV:2 IV:3


Ped 006
Affected
Unaffected
Obligate Carrier
Possibly Affected
Examined Clinically
Normal TDT (Z < 2.5)
Abnormal TDT (Z > 2.5)
I:1
I:2
II:1
II:2
9.4
II:3
2.7
II:4
-1.2
II:5
9.1
II:6
6.4
III:4 III:5 III:6 III:7 III:8 III:9
7.1
III:15 III:16
1.1
III:22 III:23 III:24 III:25 III:26
1.9
0.7
2.4
III:1 III:2 III:3
III:10 III:11 III:12 III:13 III:14
6.8 7.1 1.1 1.5 5.1
III:17 III:18 III:19 III:20 III:21
1.8 1.4 2.3 6.6


Ped 008
Affected
Unaffected
Obligate Carrier
Possibly Affected
Examined Clinically
Normal TDT (Z < 2.5)
Abnormal TDT (Z > 2.5)
I:1
I:2
II:1
II:2
II:3
II:4
II:5
II:6
II:7
III:1
III:3
III:5
III:6
-0.77
6.98
6.68
3.91
1.73
3.96
3.08
-0.82
4.07
4.9
6.98
5.37
1.88
5.89
9.80
15.95
4.95
7.82
1.52
1.78
9.07
2.25
6.26


Ped 010
Affected
Unaffected
Obligate Carrier
Possibly Affected
Examined Clinically
Normal TDT (Z < 2.5)
Abnormal TDT (Z > 2.5)
I:1
I:2
II:1
II:2
10.8
II:3
12.3
II:4
4.8
II:5
7.6
II:6
III:1
III:2
III:3
III:4
-0.7
III:5
1.9
III:6
4.4
III:7
6.8
III:8
III:9
III:10
III:11
III:12
III:13
2.9
III:14
1.7
III:15
1.8
III:16
1.7
III:17
4.4
III:18
0.5


Ped 011
Affected
Unaffected
Obligate Carrier
Possibly Affected
Examined Clinically
Normal TDT (Z < 2.5)
Abnormal TDT (Z > 2.5)
I:1
I:2
8.81
13.06
II:7
II:5
II:4
II:3
II:2
II:1
II:6
Adopted
III:8
III:7
II:6
III:5
III:4
III:3
III:2
III:1

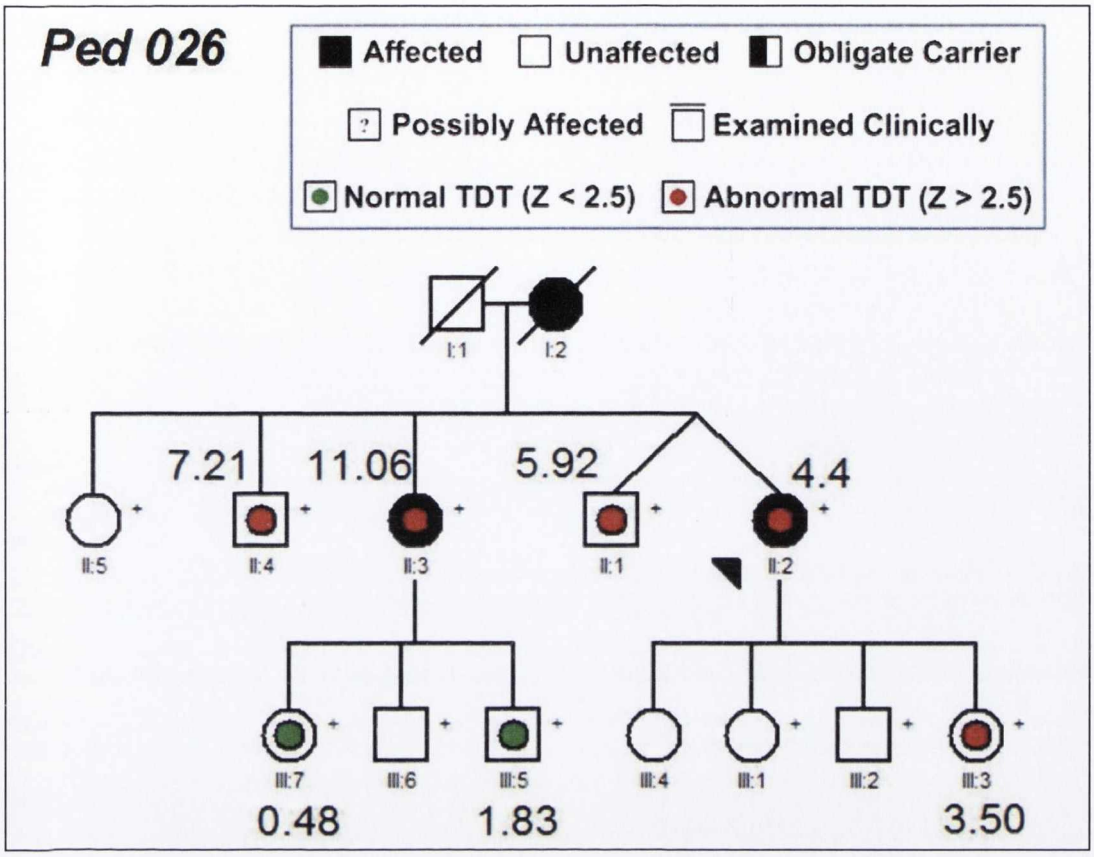


Figure 2.7: *The six familial AOPTD pedigrees (males indicated by square icons, females by circular icons). Affected individuals are represented by filled icons and obligate carriers by half-filled icons. All individuals tested for TDT have a coloured central dot (green = normal TDT, Z < 2.5; red = abnormal TDT, Z > 2.5) with individual TDT Z-Scores shown. Subjects who have been examined clinically (some of whom were not available for TDT testing) have a horizontal line above their icon.*

In a sub-pedigree of pedigree 008, the autosomal dominant transmission of the endophenotype is illustrated; IV:3 and IV:4 have abnormal TDTs and have transmitted the TDT endophenotype to their children V:5,V:7 and V:8- V:11, V:13.

In pedigree 010, the usefulness of TDT is illustrated. In addition to the four clinically affected individuals (II:3, II:5, II:6 III:13), five unaffected relatives with abnormal TDTs (II:2, II:4, III:6, III:7, III:17) are identified along with six unaffected relatives with normal TDTs who may be included in a genetic analysis.

In pedigree 006, an unaffected obligate carrier (II:2) with an affected sibling (II:6) and offspring (III:8) has an abnormal TDT (z=9.4). Both II:6 and III:8 have cervical dystonia. In this pedigree, one individual with spasmodic dysphonia (III:22) has a normal TDT (Z-Score 1.9). Autosomal dominant transmission of abnormal TDTs is demonstrated from II:3 to three of five offspring (III:10, III:11 and III:14) and from II:5 to 1 of 4 examined offspring (III:21).

DISCUSSION

The results in control subjects are tightly grouped, with TDTs generally not falling greater than 2 standard deviations from the group mean. This allows very high specificity using a cutoff of 2.5 SD to define a normal result. TDT abnormalities are not specific to dystonia, but in the case of an unaffected relative of a dystonia patient with no neurological findings, the hypothesis would be that an abnormal TDT represents non-manifesting gene carriage. This allows TDT to fulfil its eventual role in assigning gene status to relatives for genetic studies despite the fact it is a non-specific marker of basal ganglia dysfunction.

The TDT values recorded in healthy control subjects are in keeping with other published work; Hoshiyama and colleagues described a study of temporal discrimination thresholds in eighty healthy volunteers and reported a mean TDT of 26.1ms at the index finger (Hoshiyama *et al.*, 2004). Tinazzi and colleagues reported a control TDT of 35.48ms in a study of idiopathic dystonia (Tinazzi *et al.*, 1999).

The mean TDT in control subjects was lower than the range of 58 to 68 ms reported by Fiorio and colleagues (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Fiorio *et al.*, 2008). There are some methodological differences in that I chose the median for each task/side combination to attenuate practice effects and recorded at 5ms steps in my protocol.

The protocol used to measure TDT is a major determinant of performance. For example, an auditory task generally results in better performance (Grondin *et al.*, 2004). Using a more sophisticated technique, Giersch and colleagues described recording of temporal discrimination thresholds using visual stimuli with and without distracters or priming (Giersch *et al.*, 2008). They found that without distracters, the mean TDT amongst controls

was approximately 25ms while with distracters (additional lights) or primers (pre-judgement presentation of lights) the mean amongst controls rose to between 50ms and 70ms. One also must consider the fact that a subject's performance in a temporal discrimination task is affected not only by their sensory perception and central processing, but also by their motor response, and while experimental paradigms can be refined to reduce motor confounding (Stanford *et al.*, 2010), it cannot be completely excluded. Therefore, the results of studies using different protocols or equipment are not directly comparable and thresholds are only precisely applicable within a specified individual experimental paradigm.

The results of TDT testing provide convincing evidence for the role of TDT as a sensitive AOPTD endophenotype. The frequencies in AOPTD patients (73 of 81; 90%) and unaffected first degree relatives (30 of 59; 52%) are consistent with the expected ideal values of 100% and 50% respectively for an autosomal dominant endophenotype. The penetrance of the marker in patients, based on these figures, is almost complete, suggesting it can function as a marker of non-manifesting gene carriers. The finding that the rates of abnormalities are similar between cervical dystonia and writer's camp patients is supportive evidence that TDT is a relatively fundamental finding in dystonia related to basal ganglia dysfunction, rather than related to the type of dystonia expressed. This is considered in more detail in the Chapter 5.

The finding that the pattern of TDT abnormalities is essentially the same in familial patients/relatives and sporadic patients/relatives supports the hypothesis that sporadic cases are merely the only manifesting carrier of a poorly penetrant gene in their family. Again, the rates of abnormalities in sporadic relatives implies relatively complete penetrance of the marker.

The findings in second degree relatives (19 of 35 abnormal) is contrary to what one might expect for an autosomal dominant endophenotype (25% expected). This may be explained by clustering of TDT testing around the manifesting affected individuals in the pedigrees assessed rather than randomly across the pedigree.

In addition to the above, further validation of TDT as an endophenotype comes from the assessment of transmission in the multiplex pedigrees. Again, the frequencies of abnormalities in this subset of the cohort are in line with what is expected, but in addition the pattern of transmission is compatible with autosomal dominant transmission. Abnormalities are seen in all genders in all generations, with obligate clinical and endophenotype carriers found to have abnormal results and no individuals with a normal TDT having offspring with TDT abnormalities.

Finally, the rates of abnormalities in the sporadic cervical dystonia patients and their first degree relatives are encouraging when one considers the possibility of conducting a genetic study using sporadic kindreds. One option is a transmission disequilibrium technique using sibling pairs, with sample size estimates of around 100 pairs (Defazio *et al.*, 2006). This is certainly an achievable target in clinical practice and work in this area continues in the Department.

TDT FINDINGS IN THE LARGER COHORT

PATIENTS AND METHODS

The data from all subjects collected to date are included here in a repeat of the analysis set up above. 96 AOPTD patients were recruited from the cohort at St. Vincent's University Hospital to date (mean age 55.2 years, range 21.9 to -84.9). The clinical diagnosis of these patients was assessed using a videotaped neurological examination reviewed by two neurologists with expertise in dystonia. Both sporadic (no family history) AOPTD patients (n= 77) and familial (positive family history) cases (n=19) are examined. The majority (n=17) of the familial patients come from six multiplex families; the index cases of these pedigrees were DYT1 negative. The remaining patients have not had routine DYT1 screening in keeping with guidelines (Albanese *et al.*, 2006, Bressman *et al.*, 2000) as all had onset after the age of 26 years with no family history of early-onset dystonia. Several AOPTD phenotypes are represented (53 Cervical Dystonia (CD) patients, 14 Writer's Cramp (WC) patients, 11 Spasmodic Dysphonia (SD) patients, 9 Blepharospasm (BEB) patients, 1Meige's patient and 8 Musician's Dystonia Patients).

119 unaffected relatives were examined; 84 first-degree relatives (28 of familial cases, 56 of sporadic cases) and 35 second-degree relatives (all of familial cases) have been recruited to date (mean age 42.9 years; range 19.2 to 76.4). All have been examined clinically using a protocol for evidence of dystonia; none had any evidence of dystonia or dystonic tremor.

From hospital staff and visitors to the hospital, 61 healthy control subjects have been recruited to date. These were divided into two groups; under 50 years of age (n=39; mean

age 31.8 years; range 21.3 to 49.7) and over 50 years (n=22; mean age 58.7 years, range=50.3 to 71.0). Exclusion criteria include a history of neurological disease including neuropathy, visual disorder or a history of cerebral, cervical or brachial plexus injury.

SENSORY TESTING

Temporal discrimination thresholds (TDTs) were examined in a single session in a sound-proof, air-conditioned room. TDTs were measured for two tasks: (1) Visual-visual: two LED lights were used, horizontally orientated and placed on the table in front of the subject. The lights were seven degrees into the subject's peripheral vision on the side of the body being tested. (2) Tactile-tactile: Non-painful, above-threshold electrical stimulation was used on the second and third fingers on the side of the body being tested using square-wave stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom). Stimulus current was progressively increased from zero in 0.1mA steps to the lowest point at which the subject could reliably detect the impulse (tested using a paradigm with 10 trials of randomly assigned real or sham impulses requiring a response from the subject). Equality of stimulus intensity was then established between the digits if necessary. The stimulus current required ranged between 2mA and 4.5mA. Each of the two tasks was performed four times on each side of the body in random order, resulting in a total of 16 runs per subject.

The reason for the 2-task examination is based on the data presented in Chapter 5 are reviewed; essentially the mixed task was excluded from data collection after it became apparent that it did not add significantly to the results in order to refine the testing session and improve acceptability to participants of involvement in the study.

Task order was randomised to minimise practice or attention effect. Pairs of stimuli were synchronized initially and were progressively separated in 5ms steps. When the subject reported that the pairs of stimuli were asynchronous on three consecutive occasions, the first of these was taken as the TDT. The median of the four runs for each condition (2 tasks x 2 sides) was used for each subject to allow for practice effect and these four results were averaged to obtain a summary (combined) TDT score. Results of the combined TDT are shown with their standard deviation (SD) and 95% confidence intervals (CI).

ANALYSIS

The combined TDT score (the average of the results for the two task types) was used in analyses to assign status to subjects; side of body and task type were also analysed as within-subject factors. Unless otherwise stated, TDT refers to combined TDT in the results and discussion. All statistical analyses of behavioural data were conducted using Minitab 15. Groups (AOPTD patients, unaffected relatives, healthy controls) were compared using analysis of variance. Using the mean and standard deviation of the TDTs of the control group, standardised Z-scores were calculated for all subjects using the formula;

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

Z- scores of equal to or greater than 2.5 were considered abnormal.

RESULTS IN THE LARGER COHORT

CONTROL SUBJECTS

There was a statistically significant effect of age on the combined TDT score ($p<0.0023$ for slope non-zero on linear regression) (Figure 2.8). Control subjects were divided into two groups - under 50 years of age (n=39; mean age 31.8 years; range 21.3 to 49.7) and over 50 years (n=22; mean age 58.7 years, range=50.3 to 71.0) - to allow age-related normal values to be calculated.

Figure 28: *Scatterplot in all 61 control subjects, with linear regression (line) showing a statistically significant correlation between TDT (ms) and age (years) (p=0.0023).*

The mean TDT in the 39 of those less than 50 years was 24.54ms (SD 8.97 ms; 95% CI: 21.63 to 27.44 ms), (mean Z-score= 0, range -1.4 to 2.3) (Figure 2.9).

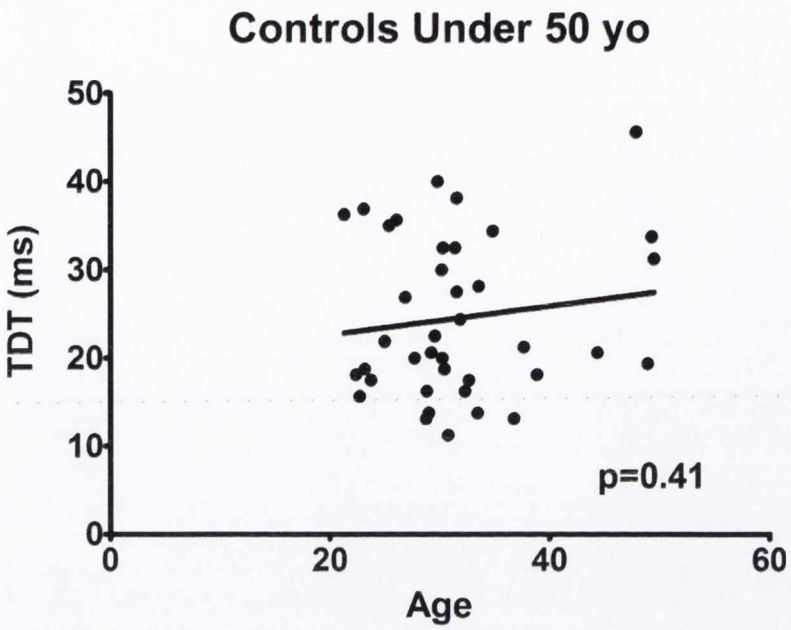


Figure 2.9: *Scatterplot of TDT results in 39 control subjects under 50 years, with linear regression (line) showing no statistically significant correlation between TDT (ms) and age (years) (p=0.41).*

In the 22 control subjects greater than 50 years of age the mean TDT was 31.11ms (SD 8.69 ms; 95% CI: 27.25 to 34.96 ms), (mean Z-score=0, range -1.5 to 2.9). In the older group, one control participant was a significant outlier with a visual TDT Z-score was 2.9, a tactile TDT Z-score of 1.71 and a combined TDT of 2.96; this result fell outside the combined TDT normal range of Z-score <2.5 (Figure 2.10). Possible explanations for this include an alternative reason for an abnormal TDT in this individual (although no relevant clinical diagnosis was present), or false positive control result (with a resulting effect on specificity of the test and the need to consider endophenocopies in studied families). All the 39 control participants less than 50 years of age had Z-scores less than 2.5. The upper limit of normal, defined as

control mean + 2.5 SD, was 46.97 ms in the under 50 group and 52.84 ms in the over 50 group.

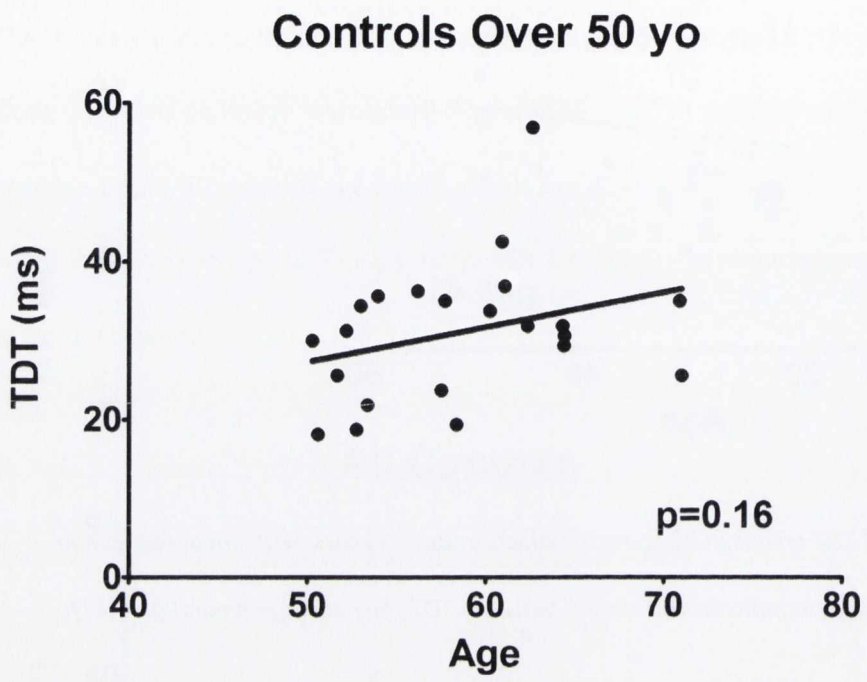


Figure 2.10: *Scatterplot of 22 control subjects over age 50 years, with linear regression (line) showing no statistically significant correlation between TDT (ms) and age (years) (p=0.16).*

AOPTD PATIENTS

80 of 96 (83%) AOPTD patients had abnormal TDTs compared to controls (Figure 2.11); the frequency of abnormalities was similar in sporadic (65/77; 84%) and familial (15/19; 79%) patients (Fisher's Exact test p=0.514). There was also a similar frequency of abnormalities when comparing the two largest groups, cervical dystonia (46/53; 87%) and focal hand dystonia (11/14; 79%) patients (Fisher's Exact Test p=0.425). The mean TDT in the patients was 73.5ms (SD 26.6 ms) (Figure 2.12).

UNAFFECTED RELATIVES

Abnormal TDTs were found in 58/119 (49%) relatives overall (Figure 2.11). The frequency of TDT abnormalities amongst the first degree relatives was 46% (39/84); the frequencies in familial first degree relatives (12/28; 43%) and sporadic relatives (27/56; 48%) were similar (Fisher's Exact Test p=0.817). 19 of 35 second degree relatives had abnormal TDTs. The mean TDT in the relatives was 49.0 ms (SD 19.9 ms) (Figure 2.12).

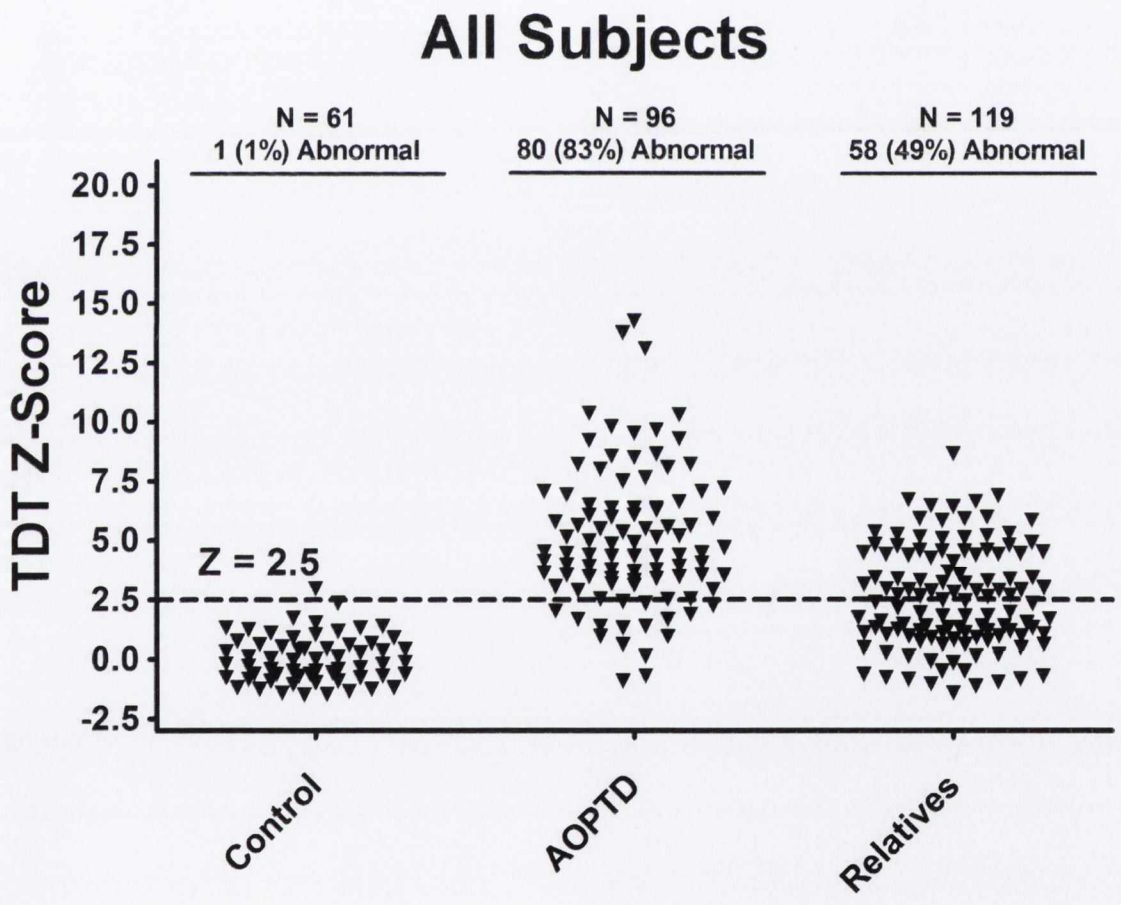


Figure 2.11: *The TDT Z-Scores in the entire cohort; 61 control subjects, 96 AOPTD patients and 119 unaffected relatives.*

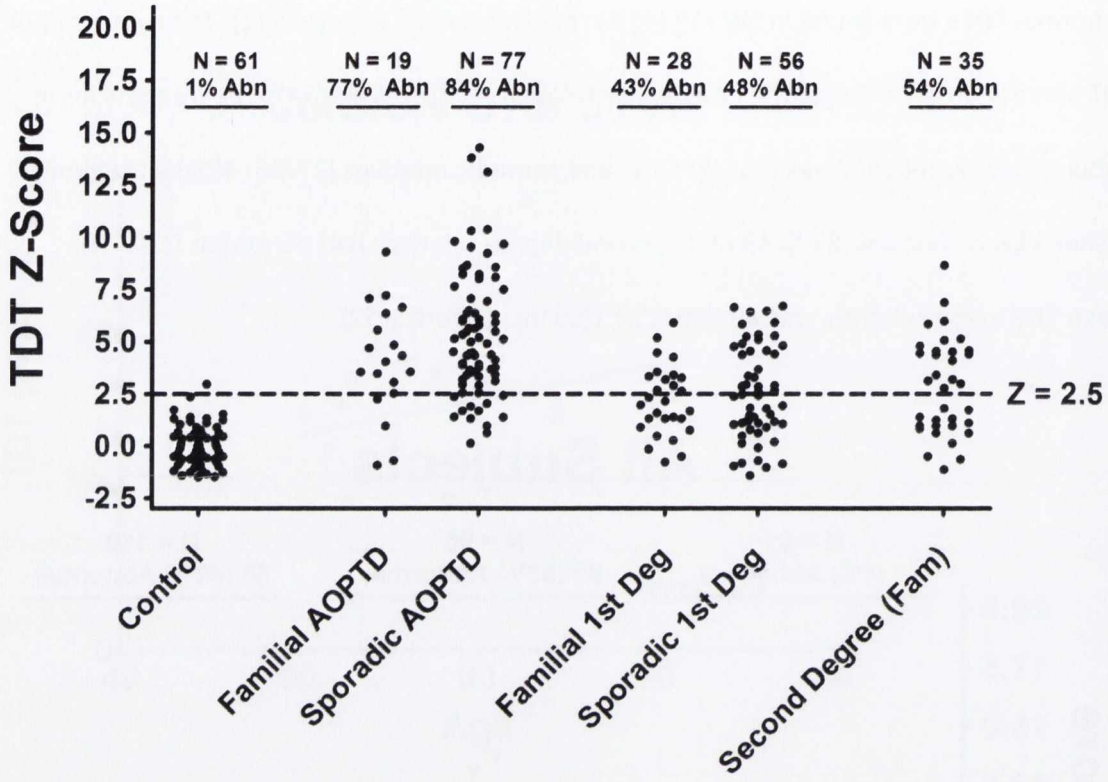


Figure 2.12: *The TDT Z-Scores in familial and sporadic patients and relatives; 19 familial AOPTD patients and 77 sporadic sporadic AOPTD patients; 28 familial first degree relatives and 56 sporadic first degree relatives; 35 second degree relatives (all familial).*

The TDTs in AOPTD patients, unaffected relatives and control subjects were statistically significantly different (1-way non-parametric ANOVA $p<0.0001$; post-hoc comparisons using Tukey 99% simultaneous confidence intervals showed that all 3 groups (patients, relatives and controls) were statistically different from each other). When analysed as a within-subject factor, side of body was non-significant.

CONCLUSION

The frequencies of TDT abnormalities in AOPTD patients and relatives are in line with an autosomal dominant endophenotype with high specificity and sensitivity. These findings are maintained as the cohort grew over the course of study. The findings across sporadic/familial cases and across two phenotypes imply that the marker is reliable and robust in AOPTD. Findings in sporadic cases support the hypothesis that all AOPTD is genetic with very low penetrance. The findings in multiplex families provide convincing evidence to appropriate transmission of TDT. The temporal discrimination threshold is likely to be a useful tool in AOPTD genetic research. The outcomes of the study described in this Chapter were published in the peer reviewed journal Brain (Oxford Journals) (Bradley *et al.*, 2009).

This Chapter has outlined the characteristics of TDT in a large cohort of AOPTD patients, relatives and controls and examined the features in sporadic and familial cases. The effect of task type and AOPTD phenotype are investigated in further detail in chapter 5. The next Chapter describes a structural imaging (voxel based morphometry) study carried out in tandem with the work described above.

CHAPTER 3
STRUCTURAL IMAGING OF TDT IN AOPTD PATIENTS AND RELATIVES: VALIDATION OF THE ENDOPHENOTYPE

Adult-onset primary torsion dystonia (AOPTD) is the most common form of dystonia; most patients appear to have sporadic AOPTD but up to 25% of these have another affected family member (Leube *et al.*, 1997, Stojanovic *et al.*, 1995). Familial AOPTD is inherited in an autosomal dominant fashion with a penetrance as low as 12-15% (Waddy *et al.*, 1991); the paucity of multiplex AOPTD families makes genetic study of the disorder difficult. The use of a sensitive endophenotype, a marker of subclinical gene carriage in unaffected relatives, is one approach to this problem.

Significant sensory processing abnormalities are found in AOPTD patients including abnormalities in spatial discrimination threshold (SDT), temporal discrimination threshold (TDT) and vibration induced illusion of movement (VIIM) (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). These sensory abnormalities may be of utility as endophenotypes. In addition, it has been proposed that abnormal sensory processing may play a primary phenomenon in AOPTD, and may play a role in the pathogenesis of AOPTD (Hallett, 1995, Tinazzi *et al.*, 2003).

As has been defined, the Temporal Discrimination Threshold (TDT) is the shortest time interval at which a subject can detect that two stimuli are asynchronous; TDT testing is

psychophysiological task that is relatively easy to administer with the advantage of showing significantly less age-dependence than other candidate sensory tests in AOPTD such as spatial discrimination thresholds (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). One study by Hoshiyama and colleagues, for example, showed little effect of age on TDT up to 65 years (Hoshiyama *et al.*, 2004). The TDT has been shown to be abnormal in DYT1 patients and non-manifesting DYT1 carriers compared to non-carrier relatives or controls (Fiorio *et al.*, 2007). The TDT has also been shown to be abnormal in patients with writer's cramp (Fiorio *et al.*, 2003), blepharospasm (Fiorio *et al.*, 2008), Parkinson's disease (Artieda *et al.*, 1992, Lee *et al.*, 2005) and multiple system atrophy (Lyoo *et al.*, 2007) and therefore may be a sensitive marker of abnormal sensory integration in the basal ganglia. An early study of temporal discrimination in subjects with focal cerebral lesions found that TDT was increased without evident sensory loss in lesions involving the putamen (Lacruz *et al.*, 1991). fMRI studies of both spatial and temporal discrimination tasks evoked basal ganglia activation (Pastor *et al.*, 2004), and during an auditory temporal discrimination task activation in the basal ganglia occurred early and was uniquely associated with encoding time intervals (Rao *et al.*, 2001). Pastor and colleagues suggested that disorders affecting the basal ganglia would affect both spatial and temporal discrimination (Pastor *et al.*, 2004).

These studies all suggest that TDT may function as an endophenotype in AOPTD by identifying subclinical basal ganglia dysfunction; however this has not been investigated by examining both AOPTD patients and their unaffected relatives. The finding that TDT abnormalities act as a marker of non-penetrant gene carriage in unaffected relatives would be useful in performing genetic studies of the disorder. The aim of this study was to validate the candidate endophenotype (TDT) by demonstrating a structural correlate associated with

abnormal TDTs in unaffected relatives using VBM. The hypothesis was that a difference in putaminal volume would be found between unaffected relatives with abnormal TDTs compared to those with normal TDTs.

VOXEL-BASED MORPHOMETRY

VBM is a structural MRI imaging method that can be used to examine structural changes at the group level. By identifying a priori a region of interested, dictated by the experimental hypothesis and pre-existing knowledge of the condition being studied, a statistical test can be applied to determine whether there is a significant different in the volume/size of a CNS region (for example the putamen) between two or more groups of subjects (typically a patient and control group).

Data are acquired by a high-resolution T1-weighted MRI sequence for each subject. The analysis consists of ***pre-processing*** the data to enable the later ***statistical analysis***. Both of these steps are carried out using academically available software, for example SPM (Wellcome Trust Centre for Neuroimaging, UCL, UK) running under Matlab 6.5 (Mathworks, Sherborn, MA, USA).

PRE-PROCESSING

***Segmentation*:** The acquired images are segmented into their major components (white matter, grey matter and CSF). Movement artifact can significantly affect this process. There are a number of statistical methods to assist in accurately completing this step.

Spatial Normalisation: This step maps the individual images to a template (registration). This allows all the scans to occupy the same space in order to allow statistical analysis.

Importantly, this step retains information on the relative size of individual regions of the CNS, which if lost would make volumetric analysis between groups impossible. The template may be a standard template or derived from the mean of the experimental subjects.

Smoothing: Using an isotropic Gaussian kernel, the grey and white matter are smoothed. The main function of this is to compensate for bias/inaccuracies introduced by automated image normalization which facilitates parametric statistics. The size of the kernel is generally determined by the size of the expected differences between the groups being studied.

Modulation: This also predominantly addresses potential distortion introduced by normalization. This step aims to account for errors in total grey matter estimation caused by individual volumetric brain differences. As alluded to above, this allows the relative size of brain structures within and between subjects to be retained despite mapping to a template of constant absolute size. A value is assigned to denote the concentration or density of voxels for a given region depending on how much absolute size change occurred during the normalization of that individual's scan. (e.g. if the absolute size of an individual scan was twice the size of the template, and therefore halved during normalization, the assigned density is doubled during modulation, and the relative size of structures within that individual is retained).

ANALYSIS

Factors such as total grey matter volume, age, sex and handedness are typically entered as nuisance factors in VBM analysis. Pre-processed data are entered into SPM and a region of interest defined using an anatomical mask (e.g. using the Wake Forest University PickAtlas

(Maldjian *et al.*, 2003). This software employs SPM5's small volume correction feature, reducing the number of multiple comparisons. Type I errors were controlled using False Discovery Rate (FDR) of 0.05, which controls the expected proportion of false positives among supra-threshold voxels for each analysis performed (Genovese *et al.*, 2002). The locations of significant voxels were summarized by their local maxima separated by at least 8 mm, and by converting the maxima coordinates from MNI to Talairach coordinate space. These coordinates were assigned neuroanatomic labels using the Talairach Daemon brain atlas (Lancaster *et al.*, 2000).

VOXEL BASED MORPHOMETRY IN DYSTONIA

It is commonly held that standard imaging is completely normal in primary dystonia. To date, several studies have examined MRI structure in AOPTD and other forms of dystonia, using various research techniques, in order to explore pathophysiology. The earliest references to such studies date from 1994, when Schneider et al reported that "high-field" (at the time 2-tesla) MRI revealed prolonged calculated T2 times in the bilateral putamen and pallidum in 17 idiopathic cervical dystonia cases compared to 28 healthy controls; no difference was noted in volume (using standardised visual quantification) or signal intensity ratios (Schneider *et al.*, 1994).

Subsequently, putaminal volume was examined in 1998 when Black et al. compared 13 AOPTD patients to 13 matched control subjects. They initially employed a mathematical stereologic method and then repeated the study with a second blinded rater who used a manual tracing technique with direct voxel counting. They demonstrated that the putamen was 13% larger in the patients using the first method and 8% (non-significant p=0.06) using the second (Black *et al.*, 1998).

Since the debut of voxel-based morphometry in 1995, applied to schizophrenia patients (Wright *et al.*, 1995), several studies have examined AOPTD patients using the method. In comparing 10 cervical dystonia patients to 10 healthy controls, Draganski et al. reported patients to have significantly higher grey matter density in the motor cortex and cerebellar flocculus bilaterally and unilaterally in the right GPi, and significantly lower density in the right caudal supplementary motor area the right dorsal lateral prefrontal and visual cortex (Draganski *et al.*, 2003). A further VBM study demonstrated higher grey matter density in the bilateral primary sensory and motor cortices in the regions representing the hands in 36 patients with unilateral focal hand dystonia compared to 36 healthy controls (Garraux *et al.*, 2004). Etgen et al. examined 16 blepharospasm patients compared to 16 controls and found bilateral putaminal enlargement in the patients, not correlated with disease duration or botulinum toxin treatment duration; they also reported decreased grey matter density in the left inferior parietal lobule correlated with duration of botulinum toxin use (Etgen *et al.*, 2006). The next year, Obermann et al published another VBM study in cervical dystonia and blepharospasm patients, where they found that CD patients had reduced putaminal volume with increased grey matter in the thalamus, caudate head bilaterally, superior temporal lobe, and left cerebellum (Obermann *et al.*, 2007). They further showed blepharospasm patients also had decreased putaminal volumes with increased caudate and cerebellar volumes, but in contrast to CD there was reduced thalamic grey matter density. Delmaire et al used VBM to examine the cerebrum, subcortex and cerebellum in 30 writer's cramp patients (Delmaire *et al.*, 2007). They demonstrated that decreased GM volume was present in the dominant (contralateral) sensory and motor cortices, and the bilateral thalami and cerebellum, implying disruption of wide-ranging CNS sensory and motor circuits, and specifically consistent with the hypotheses of abnormal plasticity in focal hand dystonias or

abnormal cerebellar motor influence. Previous work in my own Department has examined VBM findings in unaffected relatives of sporadic and familial AOPTD patients, comparing those with normal and abnormal spatial discrimination thresholds (STDs). STD is a candidate endophenotype of non-manifesting AOPTD gene carriage. This analysis showed decreased caudate and increased sensory cortex grey matter density bilaterally in familial relatives with abnormal STDs and also decreased putaminal volume bilaterally in sporadic relatives with abnormal STDs, linking a candidate endophenotype to striatal and cortical morphological changes (Walsh *et al.*, 2009). More recently, a longitudinal follow-up study of grey matter volumes in cervical dystonia has been published, demonstrating that the decreased density in left caudate, putamen and in the bilateral premotor and primary sensorimotor cortices seen in an initial study was still present at 5 years, and more prominent in the sensorimotor cortices (Pantano *et al.*, 2011).

A study in generalised dystonia (n=9), cervical dystonia (n=11) and focal hand dystonia (n=11) by Egger et al attempted to define structural MRI changes common to all forms of idiopathic dystonia. They reported a bilateral increase in grey matter density in the GPi, nucleus accumbens, prefrontal cortex, and a unilateral increase in the left inferior parietal lobe comparing the entire dystonia group to controls (Egger *et al.*, 2007). They admitted that increased GPi volume was not significant in the groups separately (borderline in the CD group) but point to sample size in each group as a limiting factor. They further reported borderline significant increased grey matter volume in the orbitofrontal cortex, right medial frontal cortex, left supplementary motor area and left cingulate gyrus in the CD cohort only. These results suggested that there may be structural abnormalities common to all AOPTD

patients as well as others that are specific to particular phenotypes, but the sample size prevented a definitive analysis of these considerations.

In order to examine genotype-phenotype interaction, Draganski et al carried out a further large VBM study in 22 DYT1 carriers (11 manifesting, 11 non-manifesting), 15 DYT1-negative adult-onset primary dystonia patients (mixed) with a family history, 14 DYT1-negative adult-onset primary dystonia patients (mixed) without a family history ("sporadic") and 28 healthy controls (DYT-negative, asymptomatic, no family history of dystonia) (Draganski *et al.*, 2009). They found that both control subjects and symptomatic DYT1 carriers had smaller putamina than the other groups, and postulated a dystoina gene effect on structure (enlarged putamen) and a disease manifestation effect (reduced putaminal volume) in DYT1, not seen with the other (non-DYT1) primary dystonia patients. A secondary finding was that within the DYT1 manifesting patients, there was a correlation between dystonia severity and putaminal volume, with volume being smaller in those with more severe disease. These findings lead some interesting considerations. It may be that there are fundamental differences between DYT1 and AOPTD, with the former more resembling a neurodegenerative process (McNaught *et al.*, 2004). Alternatively, it may be that in asymptomatic carriers, the DYT1 gene is lacking additional environmental or intrinsic factors (e.g. aberrant plasticity) that are required for disease manifestation to occur, and that the presence of these factors in the DYT1 manifesting patients, rather that the dystonia itself, results in smaller putaminal volumes, among other things. Another possible explanation may be that the enlargement seen in non-manifesting DYT1 carriers is compensatory, and is lost (or never present) in those manifesting disease; the reason for its presence in manifesting AOPTD patients may be that there is a form of compensation occurring, limiting

their dystonia (focal vs. generalized). A further suggestion offered by the authors is that putaminal enlargement in the AOPTD groups is a fundamental, pathogenic abnormality, borne out by the fact that there is no correlation with severity in that group in the study. Lastly, they suggest that the decreased putaminal volume in DYT1 manifesting patients and not in the AOPTD patients may be related to younger age at onset (15.5 years vs 36-42 years) and with longer duration of disease, with possible differential effects of dystonia genes on putaminal volumes at different ages and also longer duration of aberrant motor and sensory function in the DYT1 patients compared to AOPTD. This important paper reports a number of important observations and highlights a number of outstanding questions and avenues for future research (Draganski *et al.*, 2009).

Structure-function correlations have also been examined; Granert et al recently published a study of 14 writer's cramp patients in which both VBM studies and a measure of cortical excitability (resting motor threshold using transcranial magnetic stimulation) were acquired to compare the effects of an initial period of immobilization to a subsequent period of training (Granert *et al.*, 2011). After the initial 4 weeks of immobility, a decrease in contralateral M1 (motor cortex) volumes was demonstrated along with a decrease in cortical excitability. These changes were reversed after the subsequent 4 weeks of training with increased M1 cortical volume and increased excitability above baseline levels at the completion of the study. This demonstrated marked structural changes in response to motor training, mirrored by a functional measure of cortical excitability, suggesting a significant plasticity-related response to this form of intervention for focal hand dystonia. A further structure-function correlation study was carried out by Simonyan et al recently in 40 spasmodic dysphonia patients (Simonyan and Ludlow, 2011). This demonstrated increased

GM volumes in the laryngeal sensorimotor cortex, inferior frontal gyrus, superior and middle temporal and supramarginal gyri, and in the cerebellum. These areas also showed increased fMRI activation and cortical thickness. In some regions there was a correlation with SD severity (cerebellum and inferior frontal gyrus) and duration (inferior frontal gyrus cortical thickness only). Lastly, a VBM study in musicians examined both structure and function while playing scales (Granert *et al.*, 2011). Grey matter volume in the bilateral mid putamen (associative motor territory) was lower in pianists with greater skill playing scales. Musician's dystonia patients as a group had increased right mid putaminal volumes compared to unaffected Musicians. They conclude that while smaller putaminal volume can be shown to be associated with greater skill in pianists, relative putaminal expansion is a marker of Musician's dystonia in their cohort.

Taken as a whole, these studies report some relatively consistent findings i.e. that dystonia patients have bilateral putaminal enlargement and sensorimotor region enlargement. Also reported relatively frequently is enlargement in cerebellar regions. However, some contrary results are also seen in studies of comparable power and subject composition. These studies demonstrate that cortical change is an important feature of idiopathic adult-onset focal dystonia, but highlight the fact that structural imaging findings conflict at times, and replication studies along with functional (imaging and neurophysiology) correlation are required to refine these data.

PATIENTS AND METHODS

The work carried out in this study was carried out in collaboration with Dr. Robert Whelan, Trinity Centre for BioEngineering, Trinity College Dublin.

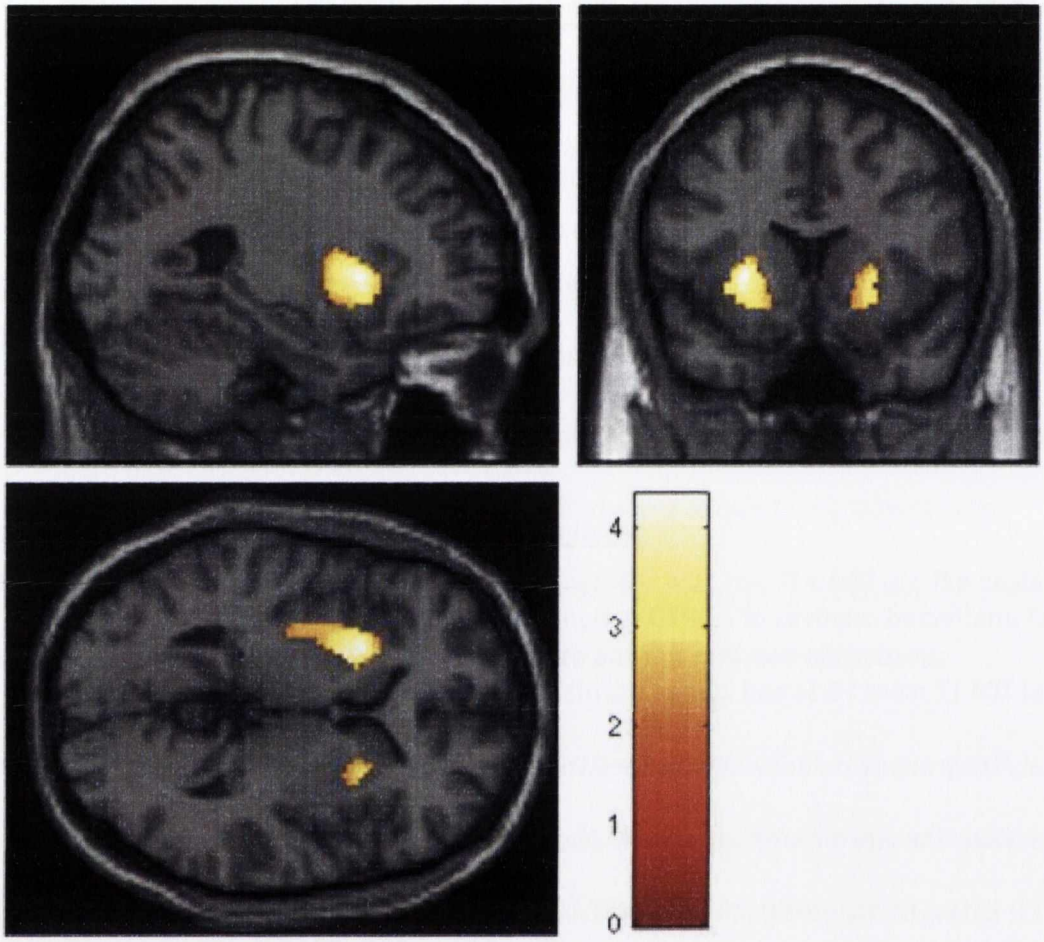
Structural MRI was acquired in 33 relatives (13 first-degree sporadic relatives, 11 first degree familial relatives, 9 second-degree familial relatives). All MRI scans were obtained at 1.5 T on the same scanner (Siemens Avanto, Erlangen, Germany). A high-resolution three-dimensional T1-weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence was acquired (TR = 1160 ms; TE = 4.21 ms, TI = 600 ms, flip angle = 15 °) with a sagittal orientation, a 256x256 matrix size and 0.9 mm isotropic voxels.

Statistical parametric mapping software (SPM5; Wellcome Centre for Neuroimaging, London, UK), running under Matlab 7 (Mathworks, Sherborn, MA, USA), was used to pre-process and analyze the data. Pre-processing incorporated image registration and classification into a single generative model (Ashburner and Friston, 2005). Segmented gray matter data were modulated in order to preserve volume. The spatially normalized and modulated gray matter partitions were smoothed using a 12 mm full-width at half maximum Gaussian kernel allowing parametric statistical analysis. Total gray matter volume, age and sex were entered as nuisance covariates in all analyses. Analyses were restricted to a predefined region of interest – the putamen – using anatomically defined masks (Wake Forest University PickAtlas) (Maldjian *et al.*, 2003) based on results obtained in AOPTD patients previously in the department (unpublished data). This software employs SPM5's small volume correction feature, reducing the number of multiple comparisons. Type I errors were controlled using False Discovery Rate (FDR) of 0.05, which controls the expected

proportion of false positives among supra-threshold voxels for each analysis performed (Genovese *et al.*, 2002). The locations of significant voxels were summarized by their local maxima separated by at least 8 mm, and by converting the maxima coordinates from MNI to Talairach coordinate space. These coordinates were assigned neuroanatomic labels using the Talairach Daemon brain atlas (Lancaster *et al.*, 2000).

RESULTS

Of the 33 unaffected relatives of AOPTD patients who had MRI scanning, 13 had an abnormal TDT (Z score >2.5) and 20 had normal TDTs (Z score < 2.5). The mean age of the abnormal TDT group was 41.7 years (SD)and the mean age of the normal TDT group was 38.1 years(SD). The age difference between the groups was not statistically significantly different ($t(21) = 1.11$, $p>0.05$). The mean TDT Z-score of the normal TDT group was 0.51 (range -1.83 to 2.40) and the mean TDT Z-score of the abnormal TDT group was 5.9 (range 3.39 to 12.68). Results are reported with Z value, false discovery rate p-value and Talairach x, y, z coordinates in parentheses. Relatives with abnormal TDTs had significantly greater putaminal gray matter volume compared to relatives with normal TDT in the left putamen ($Z=3.75$, $p_{FDR}=0.016$, x=-26, y=14, z=2) and right putamen ($Z=3.00$, $p_{FDR}=0.021$, x=24, y=16, z=-4), (Figure 3.1).



Statistics: p-values adjusted for search volume												
Set-level		Cluster-level			Voxel-level					x,y,z (mm)		
P	C	$p_{corrected}$	k_E	$p_{uncorrected}$	p_{FWE}	p_{FDR}	T	Z_Ξ	$p_{uncorrected}$			
0.050	2	0.029	645	0.031	0.018	0.016	4.34	3.75	0.000	-26	14	2
		0.252	131	0.300	0.139	0.021	3.30	3.00	0.001	24	16	-4

Figure 3.1: *Results of the voxel-based Morphometry (VBM) analysis (results reported with Z value, 5% false discovery rate p-value and Talairach x, y, z coordinates in parentheses) showing increased volume of the anterior and posterior putamen on the left side (Z=3.75, p_{FDR}=0.016, x=-26, y=14, z=2) and right side (Z=3.00, p_{FDR}=0.021, x=24, y=16, z=-4) in unaffected AOPTD relatives with abnormal Temporal Discrimination Thresholds (TDTs) in comparison to relatives with normal TDTs.*

DISCUSSION

The novel finding of bilaterally increased putaminal volume when comparing asymptomatic relatives with abnormal TDTs to those with normal values further supports and validates the endophenotype. Increased putaminal volume has been reported in manifesting AOPTD patients including those with idiopathic blepharospasm (Etgen *et al.*, 2006), focal hand dystonia and cranial dystonia (Black *et al.*, 1998) and as discussed above the preponderance of evidence at present supports the view that this phenomenon is a feature of dystonia. A disease-associated phenomenon has therefore been demonstrated in individuals with the candidate endophenotype. An fMRI study of temporal processing of an auditory task showed that initial activation occurs in the striatum, particularly the putamen, followed later by more diffuse activation (Rao *et al.*, 2001), lending support to the hypothesis that the basal ganglia, and possibly dopaminergic pathways in particular (Malapani *et al.*, 1998), act as a basic time processor in the CNS. Further fMRI studies have confirmed the central role of the putamen in temporal processing (Nenadic *et al.*, 2003, Pastor *et al.*, 2008). Interestingly, Pastor and colleagues also demonstrated that activation in the putamen decreases with perceptual difficulty suggesting it is primarily involved in automatic perception of time (Pastor *et al.*, 2008). I postulate, therefore, that a disorder of sensory integration in the basal ganglia is the patho-physiological basis of abnormal temporal discrimination in these individuals.

There are many outstanding questions relating to the multitude of abnormal experimental findings in AOPTD and whether these represent primary phenomena or secondary features

of disease manifestation (Breakefield *et al.*, 2008). This novel demonstration of increased putaminal volume in asymptomatic relatives with abnormal temporal processing is helpful in this regard. This finding suggests that putaminal enlargement is a primary phenomenon in AOPTD gene carriers and is associated with abnormal temporal processing in contrast to the suggestion that putaminal enlargement in AOPTD is secondary to abnormal dystonic motor activity (Etgen *et al.*, 2006). Further studies using temporal discrimination thresholds in AOPTD asymptomatic relatives may prove useful in defining the primary and secondary features of AOPTD. These studies could utilise fMRI or PET to measure functional processing and diffusion tensor imaging (DTI) to examine dynamic pathways.

The mean age of the relatives with abnormal TDTs was 3.7 years older than the relatives with normal TDT, a non-significant difference. The greater putaminal volume found in the abnormal TDT relatives group cannot be attributed to this difference for two reasons: age was included as a nuisance variable in the VBM analysis and the human putamen has an annual rate of shrinkage of 0.73% (Raz *et al.*, 2003).

CONCLUSION

Voxel-based morphometry further validates the hypothesis that TDT can effectively fulfil the role of a sensitive marker of subclinical gene carriage in AOPTD. The presence of increased putaminal volume in clinically unaffected relatives with abnormal TDT in this study supports the hypothesis that increased putaminal volume in AOPTD is a primary phenomenon. The results described in this Chapter were published in the peer reviewed journal Brain(Oxford Journals) (Bradley *et al.*, 2009). In Chapter 6, I examine the functional MRI features

associated with AOPTD compared to controls to further investigate the pathophysiology of the disorder. In Chapter 4, I compare TDT to Spatial Discrimination Threshold (SDT), a previous candidate endophenotype in the Department of Neurology which has some disadvantages, in order to investigate whether it is a more sensitive and reliable marker of gene carriage in AOPTD patients and non-manifesting relatives.

CHAPTER 4
COMPARISON OF TDT WITH SPATIAL DISCRIMINATION THRESHOLDS AND OTHER CANDIDATE ENDOPHENOTYPES

Chapters two and three have provided evidence to support the hypothesis that TDT acts as a sensitive marker of basal ganglia dysfunction in AOPTD patients and their non-manifesting relatives, with the findings that TDT behaves as expected in a cohort of sporadic and familial patients, is transmitted as predicted in multiplex families and validated by structural imaging findings. In this Chapter, I compare the TDT to the previous candidate endophenotype examined in the Department of Neurology; spatial discrimination threshold (SDT) testing is a method that is easy to administer but is associated with some drawbacks and had failed to significantly assist with genetic AOPTD studies. The hypothesis was that the TDT would prove a more reliable marker of abnormal sensory discrimination than the SDT.

The original description of an endophenotype dates from the early 1970s (Gottesman and Shields, 1973) when it was first applied in psychiatry to assist in the investigation of complex genetic disorders such as schizophrenia. It was proposed that the phenotypes of disorders such as schizophrenia were so variable and dependant on so many interacting genetic and environmental factors that routine evaluation of patients could never lead to successful gene identification. An endophenotype may be considered a subclinical marker of genetic liability to a disorder, whether this is determined by carriage of a single gene mutation or a number of genetic risk factors. They are biomarkers (defined as any disease-associated biological finding) that fulfil a number of specific criteria which are designed to determine

that the marker is associated with the presence of the gene rather than simply a manifestation of the disease state. The endophenotype should be associated with the disease under investigation in the general population, a heritable trait transmitted with disease in pedigrees, a finding that is "state-independent" (i.e. unaffected by disease expression or treatment) and should have a higher frequency amongst unaffected relatives in pedigrees than in the general population. Examples of endophenotypes include laboratory measurements, such as copper studies in Wilson's disease; physiological test abnormalities, for example the specific EEG findings in juvenile myoclonic epilepsy (Greenberg *et al.*, 1988); or imaging findings, including the specific pattern of MRI white matter change in CADASIL.(O'Sullivan *et al.*, 2001)

Endophenotypes could be used in linkage studies to identify genetic loci in poorly penetrant disorder; a number of criteria for a proposed endophenotype exist (Gershon and Goldin, 1986, Gottesman and Gould, 2003, Leboyer *et al.*, 1998). An ideal endophenotype for an autosomal dominant disorder should be abnormal in all affected patients, half of unaffected first degree relatives and no control subjects.

Adult-onset primary torsion dystonia (AOPTD) is the commonest form of dystonia and is considered to be a genetically determined disorder with autosomal dominant transmission (Leube *et al.*, 1997, Stojanovic *et al.*, 1995). The considerably low penetrance (in the region of 12% - 15%) results in a paucity of informative multiplex families; the majority of cases may appear sporadic in nature but up to 25% have a family history on detailed evaluation (Waddy *et al.*, 1991). Although a number of loci and genes have been identified (Muller, 2009), the genetic causes of most AOPTD phenotypes remain unknown. A sensitive endophenotype would increase the number of subjects available for genetic studies.

Sensory abnormalities in AOPTD include abnormal spatial discrimination, temporal discrimination and vibration-induced illusion of movement (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). It is hypothesised that a disorder of sensory integration possibly involving the basal ganglia is the cause of these sensory abnormalities. Given the evidence of a genetic disorder, there has been significant investigation of candidate sensory endophenotypes.

Endophenotypes have been particularly studied in DYT1 dystonia because of its incomplete penetrance, thus a potential endophenotypic trait in a can be validated in identified non-manifesting carriers of the GAG deletion in *TorsinA*. In addition, abnormalities demonstrated in non-manifesting DYT1 carriers (Carbon *et al.*, 2004, Carbon *et al.*, 2004, Eidelberg *et al.*, 1998, Fiorio *et al.*, 2007, Ghilardi *et al.*, 2003) support the hypothesis that the physiological abnormalities of sensory processing seen in dystonia result from genetic determinants rather than secondary changes induced by the movement disorder.

The spatial discrimination threshold (SDT) is determined using a grating orientation task employing Johnson-van Boven-Philips (JVP) domes applied to the fingertip. Abnormal SDTs have been found in AOPTD patients as well as their unaffected relatives (Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Sanger *et al.*, 2001, Walsh *et al.*, 2007). In addition to disordered sensory processing in the basal ganglia, abnormal representation in the primary sensory cortex (S1) may be important in the causation of SDT abnormalities (Bara-Jimenez *et al.*, 1998, Meunier *et al.*, 2001). Plasticity in S1 may explain some of the variability of SDT results, including improvement with botulinum toxin treatment (Walsh and Hutchinson, 2007).

The temporal discrimination threshold (TDT) is defined as the shortest time interval at which a subject can determine that two stimuli are asynchronous. Abnormal TDTs have been described in a number of conditions including DYT1-dystonia (Fiorio *et al.*, 2007), writer's cramp (Fiorio *et al.*, 2003, Sanger *et al.*, 2001, Scontrini *et al.*, 2009, Tinazzi *et al.*, 1999), blepharospasm (Fiorio *et al.*, 2008, Scontrini *et al.*, 2009), Parkinson's disease (Fiorio *et al.*, 2008, Lee *et al.*, 2005) and multiple system atrophy (Lyoo *et al.*, 2007) and as such may be an indicator of abnormal basal ganglia function. Functional imaging studies have demonstrated activation of the basal ganglia and other subcortical structures during a TDT task; higher cortical activity specific to TDT (not seen in SDT testing) was found in the anterior cingulate and presupplementary motor area, these regions may be involved in the interpretation of timing information (Pastor *et al.*, 2004). In contrast, the basic timekeeper appears to be the putamen, where the earliest activation occurs during encoding of time intervals (Rao *et al.*, 2001) and dopaminergic pathways may be particularly important (Malapani *et al.*, 1998). It has been demonstrated that easier TDT tasks induce greater putaminal activation than difficult TDT tasks (e.g. stimuli presented near the threshold for simultaneity perception) when additional areas are activated (Pastor *et al.*, 2008). In this way the putamen seems to act as the automatic time keeper in low-attention situations.

In addition to abnormal SDT and TDT, a number of other candidate endophenotypes have been investigated in AOPTD including abnormalities in vibration-induced illusion of movement (VIIM) (Rome and Grunewald, 1999), Positron Emission Tomography (PET) (Eidelberg *et al.*, 1998), Diffusion Tensor Imaging (DTI) (Carbon *et al.*, 2004), and transcranial magnetic stimulation (TMS) (Edwards *et al.*, 2003).

In this Chapter, I compare the utility of two sensory tests (SDT and TDT) as potential endophenotypes in AOPTD.

PATIENTS AND METHODS

PARTICIPANTS

Both SDT and TDT testing were performed in 24 AOPTD patients (14 cervical dystonia, 10 writer's cramp) (15 sporadic, 9 familial)(mean age 52 yrs, range 34-63 yrs) and 34 of their unaffected first-degree relatives (22 of familial and 12 of sporadic AOPTD patients) (mean age 42 yrs, range 26-69 yrs). The normal control subjects were the 141 control subjects in a published SDT study (O'Dwyer *et al.*, 2005) and the 43 control subjects in a published TDT study (Bradley *et al.*, 2009). Informed consent was obtained; the study was approved by the Ethics and Medical Research Committee, St. Vincent's University Hospital, Dublin.

METHODS

The SDT and TDT testing of individual patients and relatives was performed by two separate trained examiners (SDT by RW, TDT by DB) without knowledge of the findings of the other examiner. SDT was examined using JVP domes as described previously (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). A short description is included here;

Spatial discrimination thresholds (SDTs) were measured using a grating orientation task at the fingertips. A set of commercially available (Stoelting Co., Illinois) hemispheric acrylic domes, known as Johnson-van Boven-Phillips (JVP) domes were used. Each dome has

parallel gratings of uniform width between 0.35mm and 4.5mm. Spatial discrimination threshold was determined at the skin overlying the distal fat pad of both index fingers. The participant was seated opposite the examiner at a comfortable height and behind an opaque screen and asked to extend the index finger of the hand being tested with the nail opposed against a firm surface on the examiner's side of the screen. Beginning with the largest grating width (4.5 mm) and proceeding through progressively narrower ones, the domes were applied to the skin manually by the examiner for one to two seconds with enough pressure to indent the skin approximately 1-2 millimetres without causing discomfort to the participant. Subject performance in assessment of spatial resolution is relatively insensitive to the force of grating application and the spatial response profile and receptive field size of the afferent neurons responsible for performance is relatively independent of the indentation depth. Gratings were applied either perpendicular to or parallel to the long axis of the finger. The blinded subject was asked to identify the orientation immediately using a forced choice paradigm of 'down' or 'across' and received no feedback. There were 20 applications of each dome in a random order that was different for each hand and each subject examined. Subjects were be asked to identify the orientation of the grooves verbally before the stimulus was removed and if unsure will be asked to provide their best guess, so as to give an answer for all 20 applications given. The process continued until less than 60% of answers (12 of 20 applications) for a given grating width were correct.

The SDT for each hand was calculated by linear interpolation of the 75% level of accuracy using the formula:

$$\text{STD} = \frac{W^- + (W^+ - W^+) * (0.75 - P^-)}{(P^+ - P^-)}$$

W^+ = the largest width that scored less than 75% correct

W^- = the smallest width that achieved greater than 75% correct

P^- and P^+ are the fraction of correct responses at W^- and W^+

Subjects unable to achieve 75% correct responses for the largest (4.5mm) groove widths will be assigned an arbitrary threshold of 4.5mm and the final SDT was calculated as the mean of both hands. Care was taken to ensure that application of the domes was performed such that the direction of force application was perpendicular to the surface of the skin in order to avoid the possibility of temporal cues being supplied by the grating brushing across the skin. If there was excess sweating the fingers were dried to avoid slip of the applied domes. Individuals with excess callous formation over the surface of the skin of the index finger or who had amputation of the index finger were excluded from testing. Where cognitive impairment meant that the examiner could not be certain that the nature of the task was fully understood, these individuals were also excluded.

Temporal discrimination thresholds (TDTs) are examined in a single session in a sound-proof, air-conditioned room. TDTs are measured for three task types: (1) Visual-visual: two LED lights are used, horizontally orientated and placed on the table in front of the subject. The lights are seven degrees into the subject's peripheral vision on the side of the body being tested. (2) Tactile-tactile: Non-painful, above-threshold electrical stimulation is used on the second and third fingers on the side of the body being tested using square-wave

stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom). Stimulus current is progressively increased from zero in 0.1mA steps to the lowest point at which the subject can reliably detect the impulse (tested using a paradigm with 10 trials of randomly assigned real or sham impulses requiring a response from the subject). Equality of stimulus intensity is then established between the digits if necessary. The stimulus current required typically ranges between 2mA and 4.5mA. (3) Visual-Tactile: A combination of one LED light and stimulation of one finger on the same side is used with the same equipment. Each of the tasks is performed four times on each side of the body in random order, resulting in a total of 16 runs where 2 tasks (visual and tactile only) are used and 24 runs (where all three task types are used) per subject. Task order is randomized to minimize practice or attention effect. Pairs of stimuli are synchronized initially and were progressively separated in 5ms steps. When the subject reports that the pairs of stimuli are asynchronous on three consecutive occasions, the first of these is taken as the TDT. The median of the four runs for each condition (tasks x sides) is used for each subject to allow for practice effect and these results are averaged to obtain a summary (combined) TDT score.

For all subjects, Z-scores for SDT and TDT were calculated using the mean and standard deviation of the results in the control subjects for the test in question. To account for age effect, controls subjects were divided into 4 bands for SDT testing (20-29, 30-39, 40-49, 50-65) and 2 bands for TDT testing (under and over 50).

The Z-Scores calculated for each individual for both SDT and TDT were based on the control values from their respective age band in the control group. I defined a normal cut-off of 2.5 standard deviations above the relevant age-related control group mean. Z-Scores are calculated using the formula:

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

Z- scores of equal to or greater than 2.5 are considered abnormal.

RESULTS

SPATIAL DISCRIMINATION THRESHOLDS

The SDT Z scores in the 141 control subjects ranged from –2.06 to +2.63; one control subject exceeded the upper limit of normal. Abnormal SDTs (Z score > 2.5) were found in five of 24 (21%) AOPTD patients and in 17 of 34 (50%) first degree relatives (Figure 4.1; Table 4.1).

TEMPORAL DISCRIMINATION THRESHOLDS

All of the 43 control subjects' Z-scores were less than 2.5 (range -2.21 to +1.76). Abnormal TDTs (Z score >2.5) were found in 20 of 24 (83%) AOPTD patients and 14 of 34 (41%) of first degree relatives (Figure 4.1; Table 4.1).

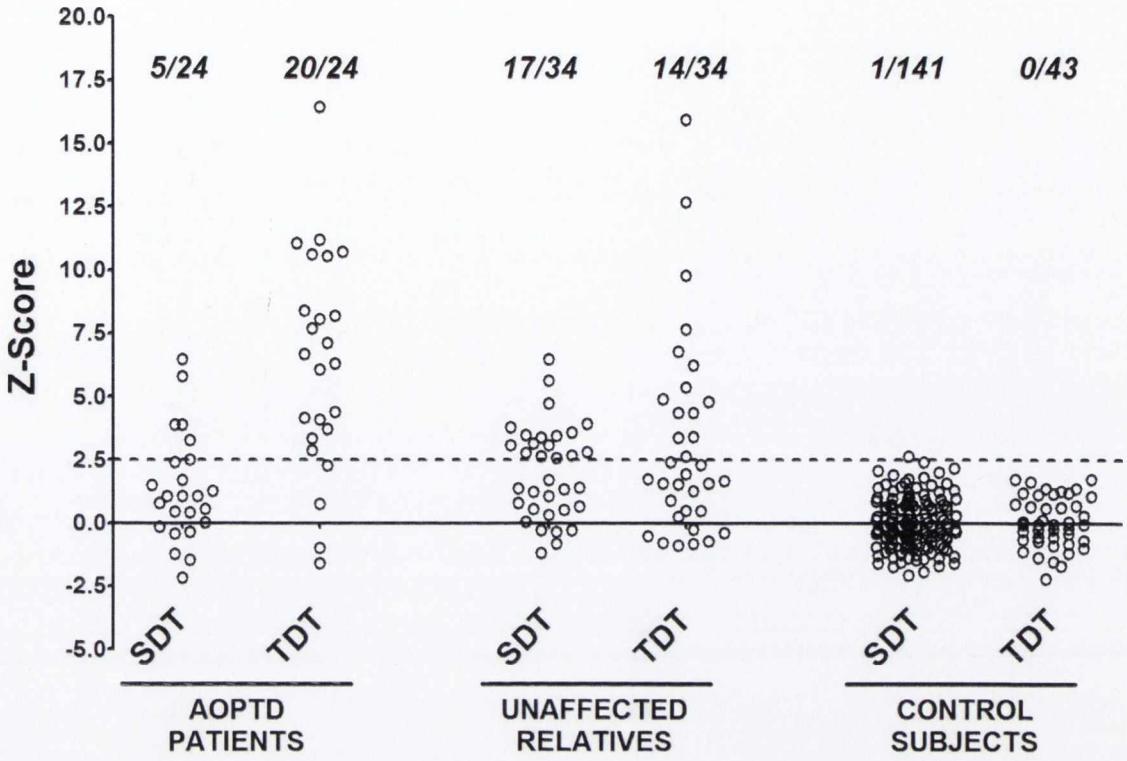


Figure 4.1: *Comparison of the frequencies of abnormalities in SDT and TDT testing. In AOPTD patients, abnormal SDTs were found in 5/24 (21%) and abnormal TDTs in 20/24 (83%). In unaffected relatives, abnormal SDTs were found in 17/34 (50%) and abnormal TDTs in 14/34 (41%). 1/141 control subjects had an abnormal SDT result and none of the 43 control subjects had an abnormal TDT. Control results for TDT were more closely grouped and the spread of abnormal results was greater than that seen with SDT testing.*

SDT (mm)	Age 20-29	Age 30-39	Age 40- 49	Age 50-65
Control Subjects Mean (SD)	1.17 (0.31)	1.05 (0.29)	1.46 (0.50)	1.85 (0.57)
Upper Limit of Normal (mean +2.5 SD)	1.96	1.77	2.72	3.30
AOPTD Patients				
Mean	N/A	2.16	2.27	2.36
(Range)		(1.42 – 2.90)	(1.01 – 3.41)	(1.00 – 4.38)
Abnormal/Tested		2 / 3	1 / 4	2 / 17
First Degree Relatives				
Mean	1.72	1.86	2.04	2.60
(Range)	(0.80 – 2.37)	(1.70 – 2.90)	(1.20 – 2.78)	(1.30 – 3.93)
Abnormal/Tested	2/4	7 / 12	5 / 11	3 / 7

TDT (msec)	**Age < 50**	**Age > 50**
Control Subjects Mean (SD)	22.85 (8.0)	30.9 (5.5)
Upper Limit of Normal (mean +2.5 SD)	42.9	44.6
AOPTD Patients		
Mean	67.1	67.5
(Range)	(15.0 – 112.5)	(22.1 – 121.0)
Abnormal/Tested	6 / 7	14 / 17
First Degree Relatives		
Mean	39.0	71.4
(Range)	(15.8 – 77.1)	(28.8 – 118.3)
Abnormal/Tested	8 / 27	6 / 7

Table 4.2: *Raw data for SDT and TDT testing in control subjects, AOPTD patients and unaffected first degree relatives are shown.*

SDT AND TDT TESTING COMPARED

In the 24 AOPTD patients there were concordant results in eight (33%) patients; in four patients both tests were abnormal and in four both tests were normal (Figure 4.2). Examining the AOPTD phenotypes separately, results were concordant in 3/14 (22%) cervical dystonia patients and 5/10 (50%) writer's cramp patients – this difference was not statistically significant (Fischer's exact test; p=0.204) (Figure 4.3). In 16 (67%) of the 24 AOPTD patients there were discordant results; one patient had a normal TDT with an abnormal SDT and in 15 patients the TDT was abnormal but the SDT was normal. In the 34 unaffected first degree relatives, 13 (38%) had concordant findings; in four patients both tests were abnormal and in nine both were normal. In 21 (62%) of the 34 relatives the results were discordant; nine relatives had an abnormal TDT with a normal SDT and 12 relatives had a normal TDT with an abnormal SDT (Figure 4.2).

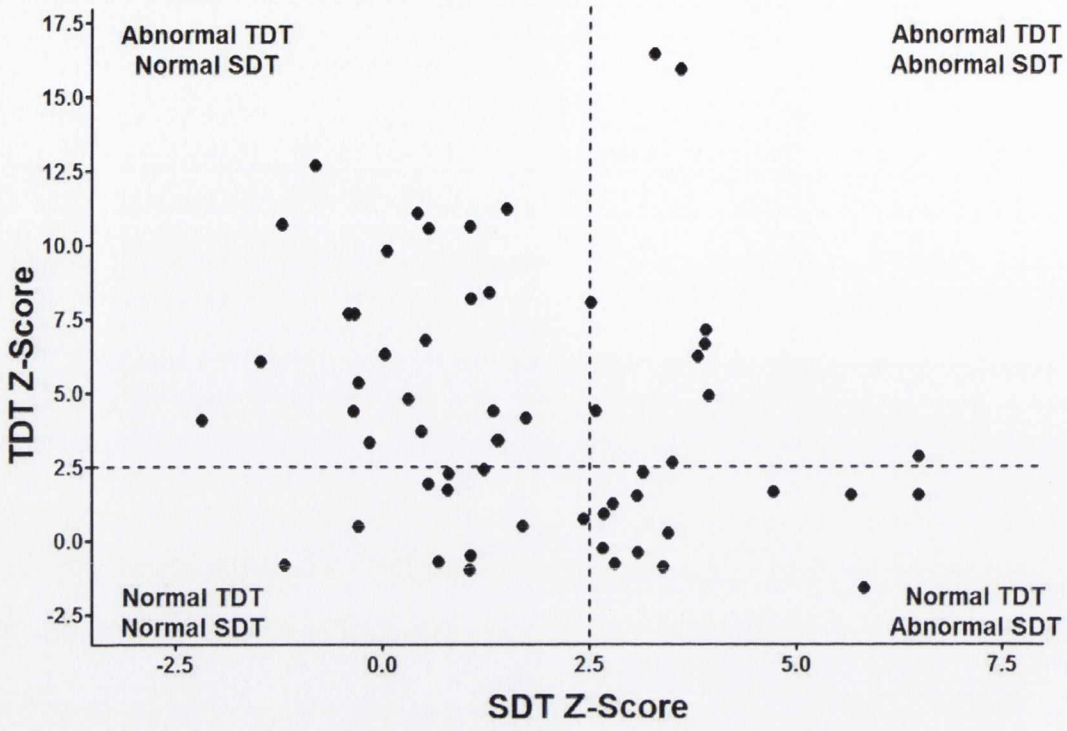


Figure 4.2: *Scatterplot of TDT Z-Score vs. SDT Z-Score in 58 subjects who had both tests (24 AOPTD patients and 34 unaffected relatives). Overall 36% (21/58) subjects were concordant (both normal or both abnormal). The majority of discordant results (24/37) represented subjects with normal SDT and abnormal TDT, possibly reflecting the lower sensitivity of SDT testing. 13 subjects had abnormal SDT results with a normal TDT and these may represent false positive SDT abnormalities.*

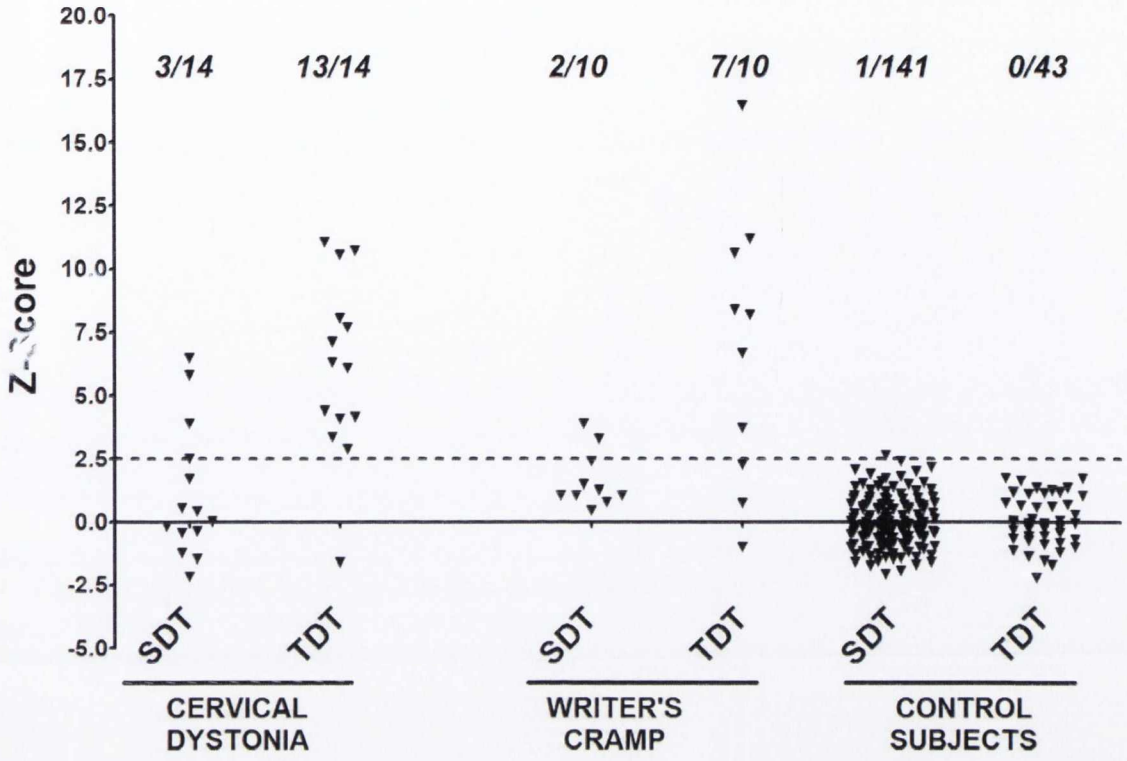


Figure 4.3: *Comparison of frequencies of abnormal TDT and SDT results in cervical dystonia and writer's cramp plotted separately.*

DISCUSSION

In the AOPTD patients I found a remarkable level of discordance (67%) between the SDT and TDT test results. In the unaffected first degree relatives, although both tests were abnormal in a significant proportion (SDT 50%, TDT 41%), there was again a remarkable discordance of 62%. Clearly one of these two potential endophenotypes is less reliable than the other. The frequencies of abnormalities in the AOPTD patients (SDT 21%, TDT 83%) indicate that TDT is a more sensitive marker of abnormal sensory processing in AOPTD. Moreover, in control subjects the distribution of TDT results was narrower (range -2.21 SD to +1.76 SD) than the

SDT control range (range -2.06 SD to +2.63SD) suggesting greater confidence that an abnormal result is indicative of abnormal central sensory processing. In addition, as can be seen from Figure 4.1, the range of abnormal Z scores for the TDT is much greater than that of the SDT. The SDT is relatively sensitive to age related changes in the peripheral nervous system; a number of discordant results may thus be due to the lower specificity of SDT testing. In addition the SDT has more potential for error due to the variability in stimuli presented to subjects using manually applied JVP domes in comparison to the electronically-determined electrical stimuli in the TDT testing procedure. The basal ganglia (Rao *et al.*, 2001), and dopaminergic pathways in particular (Malapani *et al.*, 1998), play a particular role in timekeeping in the CNS. Thus the TDT may be a more sensitive measure of the postulated dopaminergic dysfunction in AOPTD patients (Carbon *et al.*, 2009).

VALIDATION OF TDT AS AN ENDOPHENOTYPE

There is no gold standard with which to validate any candidate endophenotype in AOPTD as the genotype is not known. TDT has been examined in other genetic forms of dystonia. Fiorio and colleagues found that, at the group level, TDT was increased in DYT1-carriers (9 manifesting and 11 non-manifesting) compared to 9 non-carrier relatives and 11 healthy controls, with a mean increase of 31.2 milliseconds in non-manifesting DYT1 carriers compared to their fellow relatives negative for the gene (Fiorio *et al.*, 2007). Subsequently, in PINK1 Parkinsonism the same research group found increased TDTs in 7 homozygous manifesting patients and 14 heterozygous non-manifesting relatives compared to 14 controls (Fiorio *et al.*, 2008). That study found, using a cutoff of 2SD from the control mean, that 5/7 manifesting patients and 11/14 unaffected carriers had increased tactile TDTs (although results were non-significant using a visual task).

An ideal endophenotype for an autosomal dominant disorder should be detected in 100% of affected patients, approximately 50% of unaffected first degree relatives and no healthy controls. A review of the results for TDT, SDT and published work on other endophenotypes indicates that the frequency of abnormalities in patients, relatives and controls for TDT has frequencies compatible with a useful endophenotype in AOPTD (Table 4.2), although this should be validated in further studies in other populations.

Using Voxel-Based Morphometry, it has been demonstrated that, in comparison to relatives with normal TDTs, unaffected relatives with abnormal TDTs share a structural abnormality, bilateral putaminal enlargement, with AOPTD patients (Bradley *et al.*, 2009). Thus TDT is supported as a valid endophenotype in AOPTD by its association with a recognised pathological finding in AOPTD. Further validation of the TDT as an endophenotype comes from a study of multiplex AOPTD families in which an obligate carrier (an unaffected family member with an affected sibling and an affected child) examined by TDT had an abnormal Z score of 9.4 (Bradley *et al.*, 2009). In the same study, autosomal dominant transmission of abnormal TDTs was demonstrated in the multiplex pedigrees across two generations and no parents with normal TDTs had offspring with abnormal TDTs (Bradley *et al.*, 2009).

ALTERNATIVE ENDOPHENOTYPES IN AOPTD

Candidate AOPTD endophenotypes include abnormalities in SDT, TDT, VIIM, PET and TMS. The relative advantages and disadvantages of these techniques as potential endophenotypes have been examined in a number of patient populations (Table 4.2).

VIBRATION-INDUCED ILLUSION OF MOVEMENT (VIIM)

Vibration of a muscle through stimulation of the muscle spindle (Proske *et al.*, 1993) can induce an illusion of movement. This perception is reduced in AOPTD patients (Grunewald *et al.*, 1997, Rome and Grunewald, 1999). VIIM abnormalities were examined in a cohort of 30 AOPTD patients, 57 relatives and 19 controls (Frima *et al.*, 2008). VIIM abnormalities were found in 80% of AOPTD patients and approximately 60% of first degree relatives. As an endophenotype VIIM is not ideal, given that abnormalities were found in 21% of control subjects and thus it has a sub-optimal specificity and positive predictive value (Frima *et al.*, 2008).

TRANSCRANIAL MAGNETIC STIMULATION

Inhibitory mechanisms in the central nervous system are abnormal in patients with dystonia (Berardelli *et al.*, 1998). Transcranial magnetic stimulation has been used to assess intracortical activity in DYT1 dystonia. Edwards and colleagues studied manifesting DYT-1 patients, non-manifesting DTY1 carriers and controls (Edwards *et al.*, 2003). They reported reduced intracortical inhibition with reduced cortical silent periods in DYT1 carriers, regardless of phenotype expression, which is compatible with the reduced GABAergic activity postulated in dystonia. Impaired spinal reciprocal inhibition was however only present in manifesting DYT1 carriers. This paper desmostrated detectable abnormalities in non-manifesting gene carriers and a differential expression of abnormalities between them and their manifesting relatives. Their findings indicate a primary (probably sensori-motor integration) abnormalits associated with presence of the gene with additional features possibly secondary to gene expression or explaining clinical penetrance.

POSITRON EMISSION TOMOGRAPHY

Eidelberg and colleagues have shown using PET that metabolism is diffusely altered in DYT1 dystonia. In both manifesting and non-manifesting DTY1 carriers metabolism was shown to be increased in the lentiform nucleus, cerebellum and supplementary motor area (Eidelberg *et al.*, 1998). Additional abnormalities were seen in manifesting subjects only, including hypermetabolism in the midbrain and thalamus (Eidelberg *et al.*, 1998). Further PET studies have examined other dystonia-related mutations, including the less common DYT6 dystonia linked to 8q21-22. Compared to DYT1 dystonia, a similar network of increased metabolism was seen in DYT6 carriers, both manifesting and non-manifesting (Trost *et al.*, 2002). In an extension of this work, changes specific to DYT1 included hypermetabolism in the putamen, anterior cingulate and cerebellar hemispheres while DYT6 patients had hypometabolism in the putamen and hypermetabolism in the temporal lobe; changes specific to manifesting carriers of both genes were located in bilateral pre-SMA and parietal association cortices (hypermetabolism) (Carbon *et al.*, 2004). PET has also been used to examine motor learning in non-manifesting DYT1 carriers and reported increased metabolic activity during both sequence learning and motor execution compared to controls in the left premotor and right supplementary motor areas with hypometabolism in the posteromedial cerebellum (Ghilardi *et al.*, 2003). In this study motor output quality was similar between groups but learning reduced in the gene carriers. PET with [^{11}C] raclopride (RAC) scanning has been used to examine D2 receptor availability in the basal ganglia and selected extra-striatal regions in DYT1 and DYT6 carriers (Carbon *et al.*, 2009). The authors found that both DYT1 and DYT6 mutation carriers (affected or not) had reduced D2 receptor availability in caudate, putamen and ventrolateral thalamus compared to controls. The reduction was in

greater in DYT6 than DYT1 but within genotype there was no effect of clinical expression. The relatively consistent patterns of abnormalities relating to particular genotypes and phenotypes along with some clinical penetrance-related findings are the basis for the proposed use of functional imaging as an endophenotype in AOPTD. This modality also provides significant insight into the pathogenesis of the disorder (Carbon and Eidelberg, 2009).

DIFFUSION TENSOR IMAGING

Carbon et al describe a DTI study of manifesting and non-manifesting DTY1 patients. They found that, compared to controls, the genotype was associated with microstructural abnormalities in the connectivity of the primary sensorimotor cortex (precentral) (Carbon *et al.*, 2004). They further demonstrate that these changes were more pronounced amongst manifesting carriers, suggesting a threshold effect. They postulate that the microstructural abnormality detected in their study could be the structural basis for the well-recognised reduction of GABA-ergic intracortical inhibition in dystonia. This structural finding may represent an endophenotype in DYT1 dystonia but has not been examined in AOPTD.

Candidate Endophenotype	Affected Patients	1st Degree Relatives	Controls
IDEAL	**100%**	**50%**	**0%**
SDT	**21%**	**50%**	**1%**
TDT	**83%**	**41%**	**0%**
VIIM	**80%**	**60%**	**21%**
TMS	**Group differences reported**		
DTI	**Group differences reported**		
PET	**Group differences reported**		

Table 4.2: *Rates of abnormalities reported for various endophenotypes in AOPTD (see text for details). Only three tests have been analysed for utility as an endophenotype at the individual level in AOPTD. IDEAL = The profile of an ideal endophenotype for an autosomal dominant disorder. SDT = Spatial Discrimination Threshold testing. TDT = Temporal discrimination threshold. VIIM = Vibration-induced illusion of movement. TMS = Transcranial Magnetic Stimulation with measurement of intracortical silent periods and inhibition. DTI = Diffusion Tensor Imaging examining sensorimotor cortical connectivity. PET = Positron Emission Tomography examining metabolism in both cortical and subcortical structures.*

CHOICE OF ENDOPHENOTYPE

In many published endophenotype studies, group results are presented so that, while significant differences are demonstrated between groups of affected individuals, relatives and controls, it is not possible to be certain of the status of any one individual. TDT testing appears capable of assigning status to individuals. TDT is not without limitations; false negative and false positive results occur. In the present study four of the 24 AOPTD patients had normal TDTs. Furthermore, as part of an ongoing genetic study in the Department, it was found that removing one unaffected relative with an abnormal TDT (Z-Score 6.6) from a linkage analysis resulted in a significant increase in the logarithm of odds (LOD) score to greater than +3.0 (unpublished results). A false positive TDT was found in the control group in the a study of TDT in PINK1; one of the control subjects had a TDT greater than the

chosen cut off for normal of two standard deviations above the control mean (Fiorio *et al.*, 2008). Overall however, the number of inappropriate results seems to be low and thus specificity is relatively high. It is of critical importance that an endophenotype misclassifies the minimum number of individuals as even a few incorrect assignments in a linkage analysis can significantly affect the outcome. There are examples of this recently in the literature, including a recent reanalysis of DYT14 (thought to be a dopa-responsive dystonia due to a new locus) (Wider *et al.*, 2008). In that paper, the authors report their experience with repeat genetic analysis of a large Swiss family. An initial linkage analysis had suggested a previously reported locus at 14q13, adjacent to the known *GTP cyclohydrolase 1* (GCH1) locus known to cause DYT5 dopa-responsive dystonia. When stricter criteria were applied to disease classification (with one individual being re-assigned to phenocopy status), a second analysis revealed linkage to a larger region that now included GCH1, with a dosage method revealing a novel heterozygous deletion (i.e. DYT14 is really another DYT5 mutation). No test will be completely free of false negative results or false positives (with the need to bear in mind the possibility of endophenocopies in studied families) – as described in Chapter 2, a single control participant has been found to have an abnormal TDT during this work. In addition, while TDT appears to be relatively sensitive in detecting subclinical basal ganglia dysfunction, it is not specific to AOPTD because abnormal TDTs are seen in other basal ganglia disorders. A number of proposed AOPTD endophenotypes do not reliably dichotomize unaffected relatives to allow assignment of probable gene carriage.

CONCLUSION

Based on the available evidence TDT testing satisfies the criteria for a useful endophenotype. TDT is a more reliable marker than SDT or other currently published

candidate endophenotypes. The results in this Chapter are published in the peer reviewed journal Movement Disorders (Movement Disorders Society). In Chapter 5, I address the usefulness of TDT further by examining the utility of different task types and examining whether AOPTD phenotype affects TDT utility.

CHAPTER 5
ASSESSMENT OF TDT TASKS AND AN EXAMINATION OF TDT IN DIFFERENT AOPTD PHENOTYPES

Chapter two outlined the prevalence of TDT abnormalities in AOPTD patients, relatives and controls and also examined the prevalence of TDT abnormalities in sporadic and familial cases. In this Chapter I examine whether the task type used to measure TDT affects the sensitivity of the test and also the frequency of abnormalities in different AOPTD phenotypes. This was carried out in order to assess the most appropriate method of TDT measurement and also to examine the utility across phenotypes, with a view to practical application of the technique in genetic research.

Adult-onset primary torsion dystonia (AOPTD) is a common movement disorder associated with significant morbidity; the pathophysiology is incompletely understood. Epidemiological studies suggest that although most cases appear to be sporadic, the disorder is autosomal dominant with penetrance of 12%-15% (Leube *et al.*, 1997, Stojanovic *et al.*, 1995, Waddy *et al.*, 1991). Despite recent important developments such as the identification of DYT6 (a mutation in THAP1 associated with some adult-onset laryngeal phenotypes) (Fuchs *et al.*, 2009), progress in identifying the genetics of AOPTD has been slow.

The endophenotype approach to genetic studies in poorly penetrant disorders was first described over 30 years ago and a number candidate endophenotypes have been investigated in AOPTD (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett,

1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). The temporal discrimination threshold (TDT) is defined as the shortest time interval at which two stimuli can be determined to be asynchronous and is a promising AOPTD endophenotype (Bradley *et al.*, 2010). This sensory testing modality may be used to demonstrate abnormal temporal processing in AOPTD patients and relatives (Bradley *et al.*, 2009). and is probably a marker of basal ganglia (putaminal) dysfunction (Bradley *et al.*, 2009, Rao *et al.*, 2001) and possibly of dopaminergic pathway dysfunction in particular (Malapani *et al.*, 1998).

In this Chapter, I aimed to assess the sensitivity and specificity of three different modalities of TDT testing (visual, tactile and mixed stimuli) and to examine the ability of TDT to detect basal ganglia dysfunction (sensitivity) in a variety of AOPTD phenotypes. I hypothesized that the multimodal mixed task would be less sensitive than the other two based on a trend demonstrated in a previous study (Bradley *et al.*, 2009) and that TDT testing would be equally sensitive across AOPTD phenotypes.

PATIENTS AND METHODS

AOPTD PATIENTS

For the assessment of the task type in TDT testing, three tasks (visual, tactile and mixed) were examined in 41 patients (mean age 52, range 21 – 73 years; 25 cervical dystonia, 4 musician's dystonia, one spasmodic dysphonia and 11 writer's cramp patients). Following

the results of this initial analysis, the mixed task was omitted from the study and the visual and tactile tasks only were used for additional subjects.

For the determination of the prevalence of abnormal TDT in different AOPTD phenotypes therefore, TDT was analysed using two modalities (visual and tactile). An additional 38 patients were tested and the results for the original 41 patients were adjusted to include only their visual and tactile results. Thus a total of 79 patients were examined for phenotype analysis comprising 71 AOPTD patients [37 cervical dystonia (mean age 56.4 years), 14 writer's cramp (mean age 53.3 years), 9 blepharospasm (mean age 63.9 years), 11 spasmodic dysphonia (mean age 48.0 years)] in addition to 8 musician's dystonia patients (mean age 45.8 years). The results of the visual and tactile TDTs were averaged to give an overall measure of the subjects' temporal discrimination ability.

The diagnosis of dystonia and characterization of phenotype was carried out in a dedicated dystonia clinic by two neurologists with expertise in movement disorders. A subset of these patients (n=29) have been reported in previous studies from our group (Bradley *et al.*, 2010, Bradley *et al.*, 2009).

CONTROL PARTICIPANTS

51 healthy control subjects were recruited from hospital staff and visitors to the hospital. Exclusion criteria were a history of neurological disease including neuropathy, visual disorder or a history of cerebral, cervical or brachial plexus injury and a family history of dystonia. Control subjects were divided into two groups; under 50 years of age (n=34; mean age 31 years; range 22-49) and over 50 years of age (n=17; mean age 58 years, range=50-71). This resulted in 2 control groups, within which there was no correlation between age

and TDT result, allowing standardised Z-Scores to be calculated as described below. All controls performed all three tasks and their results (either for all 3 tasks or the visual + tactile tasks only) were used in analyses as appropriate.

SENSORY TESTING:

TDT testing was carried out as described previously (Bradley *et al.*, 2009). Briefly, in the comparison of TDT task type, subjects were tested for three modalities; a visual task (two flashing LED lights), a tactile task (non-painful electrical stimulation of the index and middle finger) and a mixed task (one LED light and electrical stimulation of one finger). Tasks were performed four times on each sided of the body resulting in a total of 24 runs (or 16 runs where only visual and tactile were carried out). The results were then averaged to determine the overall TDT. For the comparison of phenotypes, two modalities were tested (visual and tactile) using the same experimental protocol.

RESULTS

COMPARISON OF TASKS

Abnormal visual TDTs were found in 35/41 (85%) of AOPTD patients, abnormal tactile TDTs in 35/41 (85%) and abnormal mixed TDTs in 26/41 (63%) (Figure 5.1). There was a significant difference between the reduced frequency of abnormal TDTs using the mixed task compared to either of the other two modalities (Fisher's exact test P=0.041). The pattern of reduced rates of abnormal TDT tests in the mixed task held when subjects were divided into younger and older age groups (under 50 years: visual 79%, tactile 71%, mixed 42%; over 50

years: visual 89%, tactile 92%, mixed 74%), although patient numbers in these subgroups were insufficient to allow testing for statistical significance. In the 51 control subjects, the mean mixed TDT was greater than either the visual or tactile TDT (25.7 ms for mixed compared to 23.5 ms and 23.8 ms for visual and tactile under 50 years; 33.2 ms for the mixed task compared to 30.2 ms and 29.3 ms for visual and tactile over 50 years). In addition, the standard deviation was greater for the mixed task under 50 years (Table 5.1). There was relatively good correlation between raw results for visual and tactile TDT testing when examined across the 51 control subject sand 41 AOPTD patients with an r^2 of 0.71 (Figure 5.2).

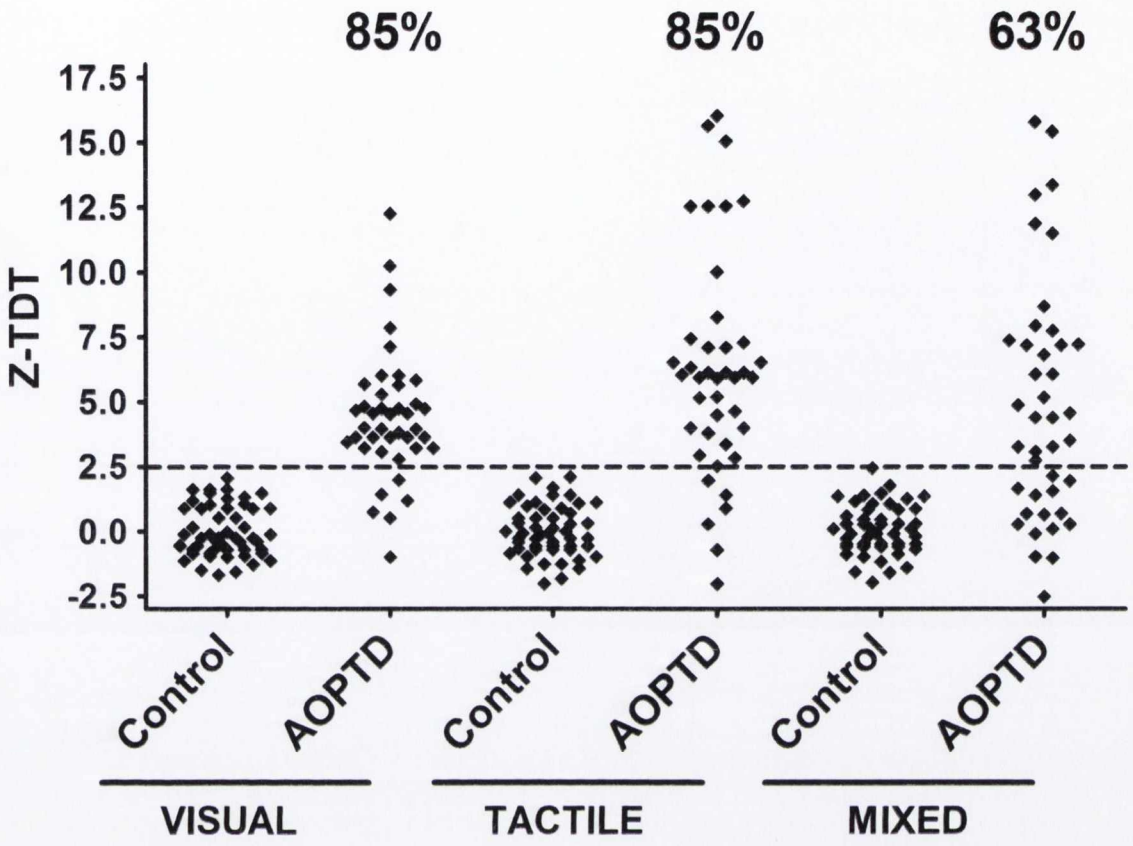


Figure 5.1: *TDT Z-Scores for three TDT modalities (visual, tactile and mixed) in 51 healthy control subjects and 41 AOPTD patients. The rates of abnormal TDTs were similar (85%) using the visual and tactile tasks; the mixed task was less sensitive at 63% (p=0.041)*

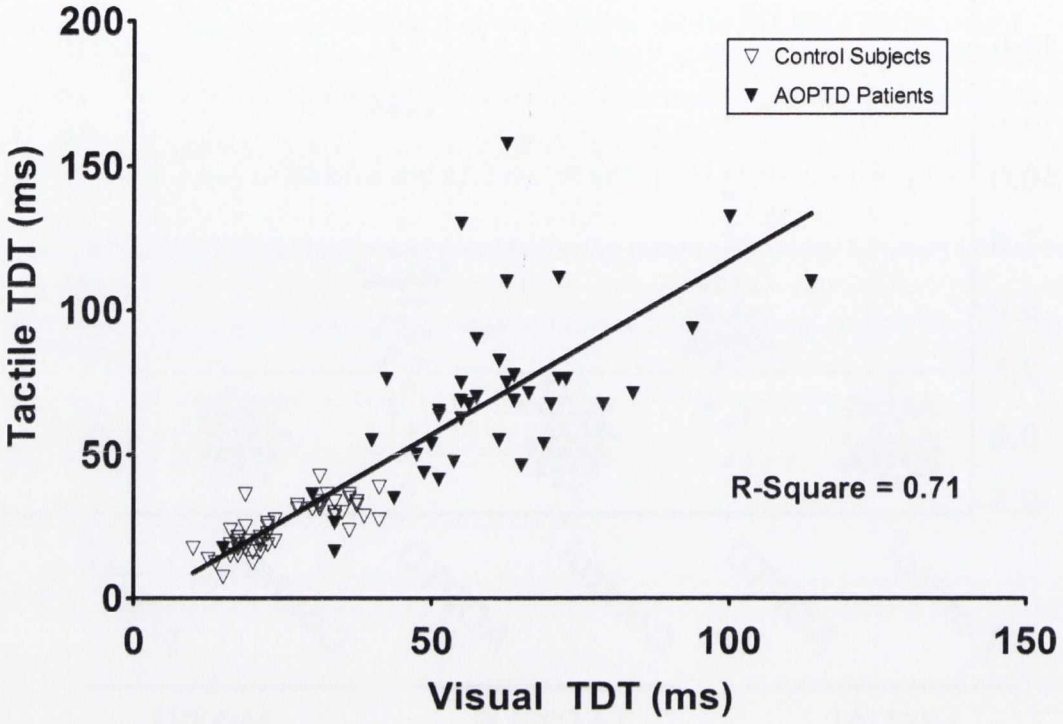


Figure 5.2: *Scatter plot of raw visual vs. tactile TDTs (ms) in 51 healthy control subjects (empty symbols) and 41 AOPTD patients (filled symbols) demonstrating the correlation between the two tasks.*

COMPARISON OF PHENOTYPES

Abnormal TDTs (using visual and tactile modalities) were found in 36/37 (97.3%) cervical dystonia (CD) patients, 12 of 14 (85.7%) writer's cramp (WC) patients, eight of nine (88.8%) blepharospasm (BEB) patients, ten of eleven (90.1%) spasmodic dysphonia (SD) patients and five of eight (62.5%) with musician's dystonia (Figure 5.3, Table 5.1). There was no statistically significant difference between the first four phenotypes tested (CD, BEB, SD, WC) but the frequency of abnormal TDTs was significantly lower in Musician's dystonia patients when compared to the other phenotypes grouped together (Fisher's exact test p=0.03).

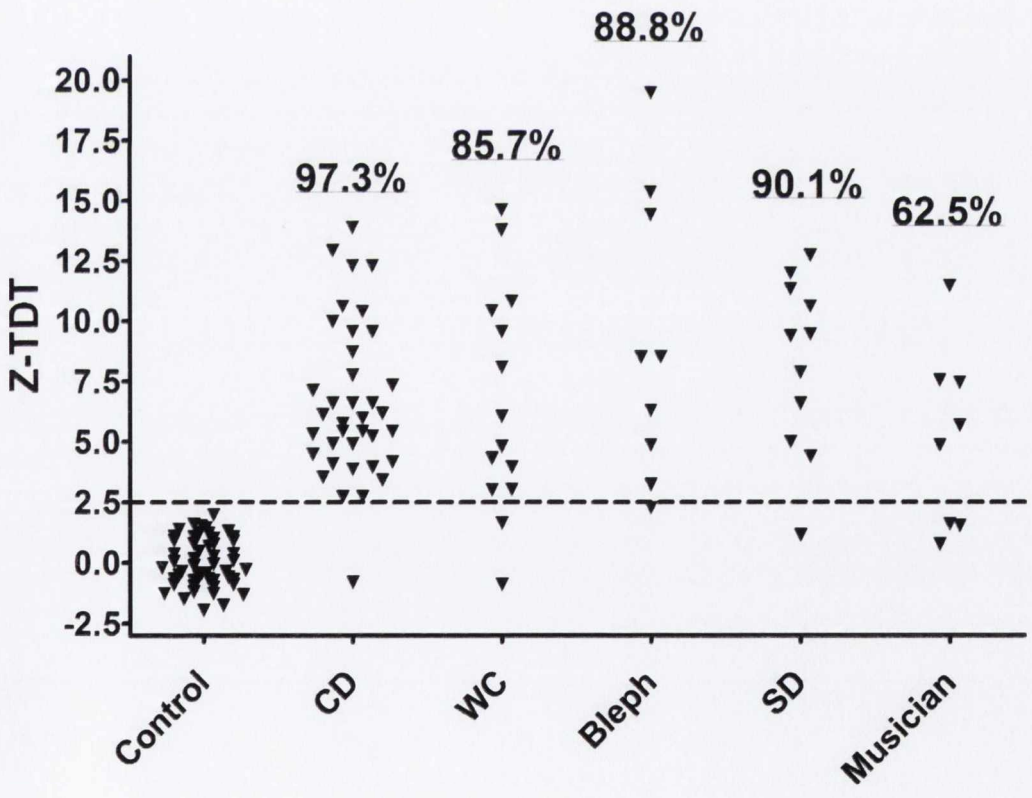


Figure 5.3: *TDT Z-Scores in 51 healthy control subjects and in the AOPTD phenotypes: Abnormal TDTs were found in 36 of 37 (97.3%) cervical dystonia (CD) patients, 12 of 14 (85.7%) writer's cramp (WC) patients, 8 of 9 (88.8%) blepharospasm (Bleph) patients, 10 of 11 (90.1%) spasmodic dysphonia (SD) patients and 5 of 8 (62.5%) of musicians tested.*

	N	Mean TDT (ms)	SD (ms)	Mean Z-Score	Range Z-Score
Control <50 All Tasks	**34**	**23.7**	**8.3**	**0.0**	**-2.0 to 2.0**
- Visual Task	34	23.5	8.6	0.0	-1.3 to 2.1
- Tactile Task	34	23.8	8.9	0.0	-1.2 to 2.1
- Mixed Task	34	25.7	11.8	0.0	-1.6 to 2.2
Control >50 All Tasks	**17**	**29.7**	**5.9**	**0.0**	**-2.0 to 1.2**
- Visual Task	17	30.2	6.8	0.0	-1.7 to 1.6
- Tactile Task	17	29.3	6.4	0.0	-2.0 to 1.1
- Mixed Task	17	33.2	6.7	0.0	-2.0 to 1.8
ALL AOPTD	**123**	**72.5**	**24.8**	**6.9**	**-0.9 to 19.5**
- Cervical Dystonia	37	67.6	18.7	6.6	-0.8 to 13.9
- Writer's Cramp	14	71.2	30.7	6.9	-0.9 to 14.6
- Blepharospasm	10	84.8	37.8	9.2	2.3 to 19.5
- Spas. Dysphonia	11	83.2	24.1	8.2	1.1 to 12.7
Musicians	**8**	**55.1**	**19.8**	**4.2**	**0.8 to 7.5**

Table 5.1: *The raw TDT results with standard deviations (ms) along with mean and range of Z-Scores in the two control groups, the four AOPTD groups with combined results and the Musicians.*

DISCUSSION

In this study, I have found that the mixed visual-tactile task was significantly less sensitive than pure visual or tactile tasks in detecting abnormal temporal discrimination in a cohort of AOPTD patients with various phenotypes. I previously described a trend towards a mixed task being less useful (Bradley *et al.*, 2009) and in studying DYT1 patients, Fiorio and colleagues also found that the mixed task was less useful with greater spread of control results (Fiorio *et al.*, 2007). It is likely that the cross-modal nature of the task was responsible for the differences in the sensitivity of the mixed task versus the uni-modal tasks. In contrast to the uni-modal tasks, additional processing is involved when stimuli from different modalities are presented. This processing may involve additional brain regions, specific to cross-modal processing (Calvert, 2001), and therefore may introduce variability into the TDT.

Furthermore, in the case of unimodal stimuli, specifically tactile stimulation, it has been demonstrated that somatotopic, rather than spatial, separation of stimuli significantly affects the results of a simultaneity task; in comparing same site and unilateral (index and middle finger) stimulation to bilateral stimulation, better results were obtained compared to testing involving both sides, regardless of whether the fingers from both sides were adjacent or not (Kuroki *et al.*, 2010). There was no difference between same site and unilateral stimulation. In this case, inter-hemispheric neurotransmission with recruitment of area 2 of the primary somatosensory cortex, rather than area 1 alone, may explain degraded performance and is it likely that similar considerations affect the sensitivity of a multimodal TDT task.

The standard deviation of the mixed task was higher than that of the uni-modal tasks and this contributed to an increased range of the TDT scores that were considered normal. Furthermore, given the likely localization of pathology including abnormal plasticity in the striatum (Peterson *et al.*, 2010, Walsh *et al.*, 2009), tasks that draw on other centres may show less clearcut differentiation between dystonia patients and healthy controls. This has implications for the practical application of TDT in the field to recruit AOPTD patients and unaffected relatives for genetic studies. The visual and tactile modalities had equal sensitivity, thus, when simple uni-modal stimuli are used, abnormal temporal processing by the basal ganglia is reliably detected.

The finding of similar frequencies of abnormalities in cervical dystonia, writer's cramp, spasmodic dysphonia and blepharospasm patients suggests that putaminal dysfunction, reflected by the abnormal TDT, is a fundamental and state-independent disorder not related to specific phenotype or disease characteristics. This is important because the use of sporadic cases with identified non-manifesting relatives (using TDT) can in theory include families with different phenotypes to identify genetic predispositions to dystonia in general. Scontrini and colleagues found abnormal TDT results, at the group level, in some forms of AOPTD compared to control subjects and patients with hemi-facial spasm (Scontrini *et al.*, 2009) and that the body part tested did not significantly affect the finding of abnormal TDT.

TDT testing using pure visual or tactile stimuli is therefore a sensitive measure, capable of objectively identifying marked temporal processing deficits in individual AOPTD patients reflecting basal ganglia dysfunction. I propose that TDT will be of use as an endophenotype in studies of patients with all forms of AOPTD and their unaffected relatives. The five AOPTD patients (excluding musicians) who had normal TDT results are

detailed in Table 5.2: there was no consistent feature in phenotype, age or family history to suggest a subgroup in whom TDT is less useful, and at the group level no statistical differences were seen between phenotypes.

Z-Score	Phenotype	Family History	Age
-1.07	Cervical Dystonia	Familial	60
1.55	Writer's Cramp	Sporadic	60
1.28	Spasmodic Dysphonia	Familial	27
2.24	Blepharospasm	Sporadic	70
-0.82	Writer's Cramp	Familial	40

Table 5.2: *The characteristics of the five AOPTD patients (excluding musicians) who had normal TDT results.*

The results in the musician's dystonia group differ from the other patients in the study. It has been suggested that there may be a genetic basis to this disorder rather than acquisition by repetitive and highly practiced motor activity (Schmidt *et al.*, 2009). The lower prevalence of abnormal TDTs in musician's dystonia compared to focal hand dystonia suggests heterogeneity; some patients represent focal hand dystonia and others a secondary movement disorder.

CONCLUSION

TDT testing using uni-modal stimuli is a sensitive marker of putaminal dysfunction in AOPTD; multimodal techniques seem to be less reliable. TDT testing is equally sensitive across all tested AOPTD phenotypes and should therefore be a useful tool in performing genetic studies in families with all common forms of AOPTD. Musicians' dystonia behaves differently and may be heterogeneous in aetiology. TDT represents a reliable and sensitive endophenotype in ongoing efforts to identify AOPTD-related genes. The outcomes of the study presented in this Chapter is published in the peer reviewed Journal of Neurology (European Neurological Society) (Bradley *et al.*, 2011).

Following on from the results in this Chapter, it was decided to conduct further TDT testing using the visual and tactile tasks only. In Chapter 6, I examine sporadic AOPTD patients in more detail in order to examine the hypothesis that sporadic AOPTD cases represent the only manifesting person in families with a very poorly penetrant genetic condition, and the pattern of inheritance in these families to infer the most useful way to apply TDT in genetic studies.

CHAPTER 6
FURTHER INVESTIGATION OF TEMPORAL DISCRIMINATION THRESHOLDS IN SPORADIC AOPTD

The findings in Chapter 2 demonstrate that the behavioural data obtained from TDT testing is compatible with a sensitive and specific AOPTD endophenotype. It is of interest to examine sporadic AOPTD patient specifically in order to examine the hypothesis that all sporadic cases merely represent the chance occurrence that they are the only manifesting carrier of a poorly penetrant trait in their family. The natural extension of such a conclusion is the consideration of a genetic study using sporadic patients and their unaffected first degree relatives, a pool of subjects much more readily available than their familial counterparts, particularly those with multiplex families.

Adult-onset primary torsion dystonia (AOPTD) is a hyperkinetic movement disorder associated with significant morbidity and the most common form of primary dystonia. Epidemiological studies suggest that, although most cases appear to be sporadic, the disorder is autosomal dominant with a penetrance of 12%-15% (Leube *et al.*, 1997, Stojanovic *et al.*, 1995, Waddy *et al.*, 1991). Although progress in elucidating the presumed genetic basis of AOPTD has been slow, recently DYT6 dystonia, associated with some adult-onset laryngeal phenotypes, has been found to be a due to a mutation in *THAP1* (Fuchs et al., 2009). About 25% of AOPTD patients have one other family member affected (Stojanovic *et al.*, 1995), but families with sufficient numbers for linkage analysis are infrequent and most gene carriers, even in multiplex AOPTD families, are non-manifesting.

The possibility of success of linkage analysis in poorly penetrant disorders may be increased by use of an endophenotype (Gottesman and Gould, 2003, Gottesman and Shields, 1973), and a number of potential endophenotypes have been examined in AOPTD including the temporal discrimination threshold (TDT) (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). The TDT is defined as the shortest time interval at which two stimuli can be determined to be separate in time and has recently been demonstrated to be a potentially useful endophenotype in several forms of dystonia (Bradley *et al.*, 2011, Bradley *et al.*, 2010, Bradley *et al.*, 2009, Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Fiorio *et al.*, 2008, Scontrini *et al.*, 2009). However an abnormal TDT is not specific to AOPTD, having been found also in other basal ganglia disorders including in non-manifesting gene carriers of DYT1 dystonia and PINK1 Parkinsonism (Artieda *et al.*, 1992, Fiorio *et al.*, 2007, Fiorio *et al.*, 2008, Lee *et al.*, 2005, Lyoo *et al.*, 2007). Autosomal dominant transmission of abnormal TDTs has been reported in both affected and unaffected members of multiplex AOPTD families (Bradley *et al.*, 2009). The hypothesis was that most individuals with sporadic AOPTD have a genetically determined disorder and the absence of other affected family members reflects reduced gene penetrance. This study examined TDTs in sporadic AOPTD patients and their first degree relatives to determine whether TDT abnormalities are present at rates compatible with a highly penetrant endophenotype, and to investigate whether gender, relationship or age have an effect on endophenotype penetrance (factors that could have practical implications for the use of the endophenotype in the field). The hypothesis was that abnormal TDTs would be found in most AOPTD patients and approximately half of their first degree relatives.

PATIENTS AND METHODS

The research presented in this chapter was carried out jointly with Dr. Okka Kimmich, Department of Neurology, St. Vincent's University Hospital Dublin.

CONTROL PARTICIPANTS

61 healthy control subjects were recruited from hospital staff and visitors to the hospital. Exclusion criteria were a history of neurological disease including neuropathy, visual disorder or a history of cerebral, cervical or brachial plexus injury and a family history of dystonia. Control subjects were divided into two groups; under 50 years of age (n=39; mean age 31.8 years; range 21-49) and over 50 years of age (n=22; mean age 58.9 years, range=50-71). This resulted in two control groups, within which there was no correlation between age and TDT result, allowing standardised Z-Scores to be calculated as described below.

AOPTD PATIENTS

33 sporadic AOPTD patients were recruited for TDT testing (mean age 57 years, range 42-78) (31 cervical dystonia, 1 spasmodic dysphonia, 1 Meige's Syndrome). The diagnosis of primary dystonia was made at a dedicated dystonia clinic by two neurologists with expertise in movement disorders.

UNAFFECTED FIRST DEGREE RELATIVES

73 unaffected first degree relatives of the 33 sporadic AOPTD patients were examined for TDT (mean age 42 years, range 18-77) (36 siblings, 36 offspring and 1 parent). None of the unaffected relatives had any symptoms or signs of a movement disorder. Relatives were examined by the research registrars (OK and DB) and had a full medical history and

neurological examination including an examination protocol to assess for any evidence of a neurological disorder, in particular a focal dystonia. A video examination of the relatives was not performed.

SENSORY TESTING

TDT testing was carried out as described previously in familial AOPTD (Bradley *et al.*, 2009). Briefly, testing was carried out in a single session in a soundproof air-conditioned room. For the comparison of TDT task type, subjects were tested for two modalities; a visual task (two flashing LED lights) and a tactile task (non-painful electrical stimulation of the index and middle finger). Stimuli were presented at 5-s intervals and the separation between pairs of stimuli was increased in 5 ms steps. The LEDs were positioned seven degrees into the subject's peripheral field on the side being tested. LEDs were illuminated for 5 ms on each presentation of the visual stimulus. Electrical stimuli were presented using square-wave stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom) and rectangular cloth electrodes (Item # TD-141C1, Discount Disposables Post Office Box 111 St. Albans, Vermont 05478). Stimulus pulse length was set at 5 ms and stimulus current was increased (in 0.1 mA steps) until the subject could reliably detect the stimuli. Each task was performed four times on each side of the body with the median of the 4 trials in each condition (side x task) taken to eliminate practice effect. This resulted in a total of four conditions/16 trials. Thus, values in milliseconds for the visual TDT, the tactile TDT and combined visual and tactile TDT were determined.

STATISTICAL ANALYSIS

All TDT results (in milliseconds) were converted to standardised Z-Scores to enable easy comparison of individual results using the formula:

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

For each subject the Z-Score was calculated using the relevant (under 50 or over 50 years) control dataset. Z-scores were determined for each participant's visual, tactile and combined visual and tactile TDT (three Z-scores). Z-Scores of equal to or greater than 2.5 were considered abnormal. Comparison of the rates of abnormal TDTs between subgroups of relatives was carried out using Fisher's Exact Test ($p<0.05$ considered statistically significant).

RESULTS

CONTROL PARTICIPANTS

Because of an effect of age on TDT, control subjects were divided into 2 groups, over and under 50 years of age. The results of the visual, tactile and combined TDT testing are summarised in Table 6.1. The mean TDT in the 39 of those less than 50 years was 24.54ms (SD 8.97 ms; 95% CI: 21.63 to 27.44 ms), (mean Z-score= 0, range -1.4 to 2.3). Of the control participants less than 50 years of age, one subject had an abnormal tactile TDT (Z- score = 3.4) but normal visual and combined TDT results. In the 22 control subjects greater than 50

years of age the mean TDT was 31.11ms (SD 8.69 ms; 95% CI: 27.25 to 34.96 ms), (mean Z-score=0, range -1.5 to 2.9). In the older group, one control participant's visual TDT Z-score was 2.9, with a tactile TDT Z-score of 1.71 and a combined TDT of 2.96; this result fell outside the combined TDT normal range of Z-score <2.5. All the 39 control participants less than 50 years of age had combined TDT Z-scores < 2.5. (Figure 6.1; Table 6.1).

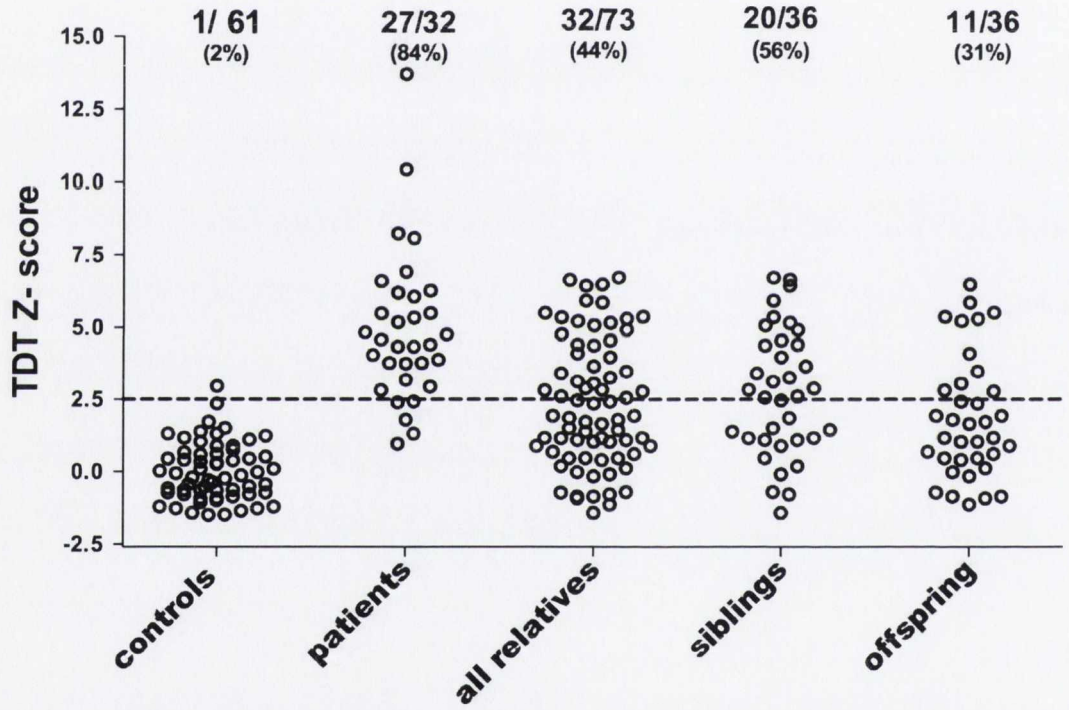


Figure 6.1 : *TDT Z-Scores in 61 healthy control subjects, 32 sporadic AOPTD patients and 73 unaffected first-degree relatives, with relatives divided by relationship to patients into siblings, offspring to the right of the graph. (Z-TDT: Z-score of the temporal discrimination threshold, AOPTD: adult onset primary torsion dystonia).*

AOPTD PATIENTS

One of the AOPTD patients with cervical dystonia was unable to complete the TDT task because of visual impairment; her relatives were examined. In the other 32 AOPTD patients abnormal visual TDTs were found in 25/32 (78%). Only 29 of the 32 AOPTD patients could complete the tactile TDT task, three patients found the tactile test difficult and could not complete it (two of these three had abnormal visual TDTs); abnormal tactile TDTs were found in 24/29 (83%). Using the combined TDT 27 (84%) of the 32 AOPTD patients had Z-scores >2.5 (Figure 6.1; Tables 6.1 and6. 2). The results of TDT testing by task type are given in table 6.2. The mean combined TDT in the 32 AOPTD patients was 74.35 ms (SD 25.95 ms; 95% CI 64.99 to 83.70 ms) (mean Z-score= 4.94, range 0.96 to 13.71). Because of the higher sensitivity of using combined TDT results (84% abnormal) compared to either the visual (78% abnormal) or the tactile (83% abnormal) task alone, the Z-Score of the combined visual and tactile TDT tasks was used to ascertain the presence of abnormal TDTs in relatives.

Study subjects	TDT task	N	Mean TDT (ms)	SD	Mean Z-score	Range Z-score	Abnormal TDTs N (%)
Controls < 50 yrs	Visual	39	24.49	9.04	0	-1.6 to 1.85	0 (0%)
	Tactile	39	24.58	10.44	0	-1.6 to 3.4	1 (2.6%)
	Combined	**39**	**24.54**	**8.97**	**0**	**- 1.4 to 2.3**	**0 (0%)**
Controls > 50 yrs	Visual	22	31.08	9.67	0	-1.6 to 3.3	1 (4.5%)
	Tactile	22	31.99	11.83	0	-2.02 to 1.7	0 (0%)
	Combined	**22**	**31.11**	**8.69**	**0**	**-1.5 to 2.9**	**1 (4.5%)**
AOPTD patients	Visual	32	73.16	25.24	3.97	0.75 to 11.53	25 (78%)
	Tactile	29	73.05	27.83	5.66	1.0 to 14.69	24 (83%)
	Combined	**32**	**74.35**	**25.95**	**4.94**	**0.96 to 13.71**	**27 (84%)**
Unaffected first degree relatives	Visual	73	46.49	19.78	2.05	-1.19 to 6.56	30 (41%)
	Tactile	70	47.59	24.80	2.49	-1.84 to 10.43	26 (37%)
	Combined	**73**	**47.04**	**20.41**	**2.35**	**-1.42 to 6.7**	**32 (44%)**

Table 6.1: *The raw mean TDT results (in milliseconds) with standard deviations, mean and range of Z-scores and number and percentage abnormal of the visual, tactile and combined TDT task results for the 61 control subjects divided by age, the 32 AOPTD patients and 73 unaffected first degree relatives. (AOPTD: adult onset primary torsion dystonia, ms: milliseconds, N: number of study participants, SD: standard deviation, TDT: temporal discrimination threshold)*

UNAFFECTED FIRST DEGREE RELATIVES

The mean combined TDT in the 73 unaffected first-degree relatives was 47.04 ms (SD 20.41 ms; 95% CI 42.88 to 51.80 ms) (mean Z-score= 2.35, range -1.42 to 6.7). Abnormal TDT results were found in 32/ 73 (44%) relatives. Only one parent was tested and had an abnormal TDT, the other abnormal TDTs were in siblings [20/36 (56%)] and children [11/36 (31%)].

		VISUAL TDT		
		Abnormal	Normal	TOTAL
TACTILE TDT	Abnormal	21 *(21)*	3 *(3)*	24 *(24)*
	Normal	2 *(1)*	3 *(0)*	5 *(1)*
	Not done	2 *(2)*	1 *(0)*	3 *(2)*
	TOTAL	25 *(24)*	7 *(3)*	32 *(27)*

Table 6.2: *Temporal discrimination threshold (TDT) results in 32 AOPTD patients for the visual and tactile tasks. An abnormal TDT was defined as a Z-Score greater than or equal to 2.5 compared to the age-related control mean. The figures in brackets indicate the number of patients with abnormal combined TDT results. The combined TDT had a higher sensitivity than either the visual or the tactile task alone. Because of task difficulty three patients were unable to perform the tactile test, two of these had abnormal visual TDTs, one had a normal visual TDT. (AOPTD: adult onset primary torsion dystonia)*

FREQUENCY OF ABNORMAL TDTS IN INDIVIDUAL FAMILIES

In the 33 families the total number of siblings and children greater than 18 years of age was 250. 72 (39%) of the 189 siblings/offspring available for testing were examined. The main reason for not testing relatives was distance from the hospital laboratory. In nine families only one relative was tested (2/9 abnormal TDTs), in 15 families two relatives were tested (17/30 abnormal TDTs) and in nine families three or more relatives were tested (13/34 abnormal TDTs) (Table 6.3). When two or more relatives were tested only two of 24 families did not have an unaffected relative with an abnormal TDT.

Number of Relatives Tested in Family	Number of Families	Total Relatives Tested	Number of Relatives with Abnormal TDT	Number of Families with no Abnormal TDT
3 or more Relatives	9	34	13	0
2 Relatives	15	30	17	2
1 Relative	9	9	2	7
All families	33	73	32	9

Table 6.3*: Families of sporadic AOPTD patients divided in relation to the number of asymptomatic first degree relatives examined by TDT in each family. All families had at least one asymptomatic relative with an abnormal TDT apart from seven of nine families in which only one relative was examined and two of 15 families in which two relatives were examined. (TDT: temporal discrimination threshold, AOPTD: adult onset primary torsion dystonia)*

INHERITANCE OF THE ABNORMAL TDT ENDOPHENOTYPE

Two families illustrative of TDT transmission in sporadic AOPTD are illustrated in Figure 6.2. The frequencies of abnormal TDT in patients stratified by gender, relationship and number of relatives examined are displayed in Table 6.4 and Figure 6.3. The pattern of inheritance in the 24 families with at least two tested unaffected first degree relatives was examined (total of 64 relatives; 30 siblings, 33 offspring, 1 parent). In those families there was a significantly higher frequency of abnormal TDTs in siblings (18/30; 60%) compared to offspring (11/33; 33%) (Fisher's Exact Test p=0.0447) (Figure 6.3). In all the 73 unaffected relatives there was a trend for abnormal TDTs to be more common with age; the frequency of abnormal TDTs in relatives under 45 years was 16/44 (36%) and in relatives over 45 years was 16/29 (55%) (Fisher's exact test p=0.1496). Abnormal TDTs were found more frequently in female than male siblings and children. In families where two or more relatives were assessed, of the 30 siblings examined, 11/17 (65%) sisters and 7/13 (53%) brothers had abnormal TDTs. Of the 33 offspring examined 7/16 (44%) daughters and 4/17 (24%) sons had abnormal TDTs. Thus abnormal TDTs were found in 18/33 (51%) of sisters and daughters and 11/30 (33%) of brothers and sons (Fisher's exact test p=0.208) (Figure 3). There was no effect of gender of the propositus on transmission of abnormal TDTs. Fathers with AOPTD passed the abnormal TDT to 5/10 offspring (4/7 sons and 1/3 daughters) while mothers with AOPTD passed an abnormal TDT to 6/22 children (3/10 sons and to 3/12 daughters) (Fisher's exact test p=0.415). Five of the sporadic AOPTD patients had normal TDTs; two had only one relative tested and both these relatives had normal TDTs. Three of the five AOPTD patients with normal TDTs, who had two or more relatives tested, have relatives with abnormal TDT (three sisters and one daughter and one parent).

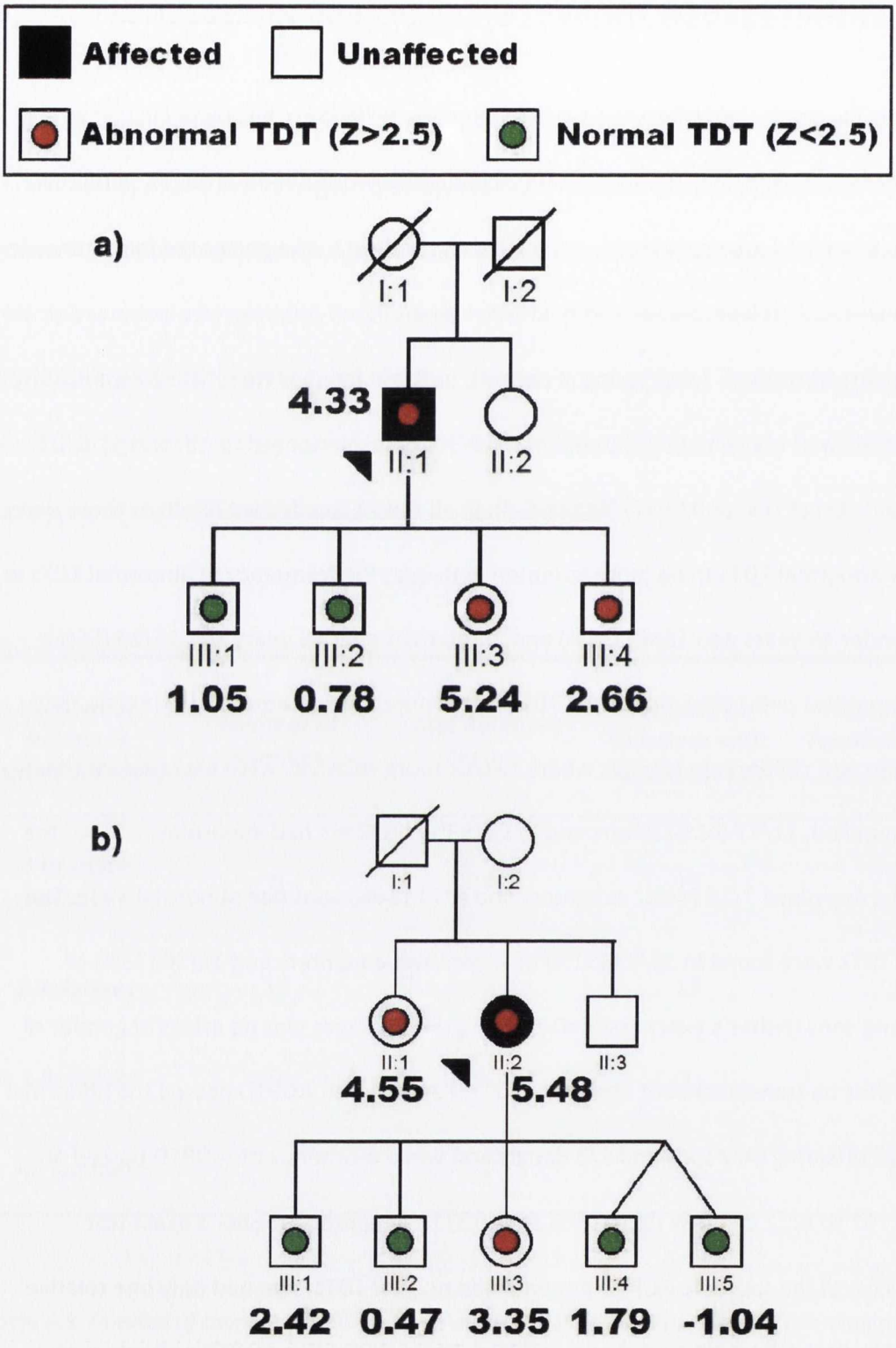

Affected
Unaffected
Abnormal TDT (Z>2.5)
Normal TDT (Z<2.5)
a)
I:1
I:2
4.33
II:1
II:2
III:1
III:2
III:3
III:4
1.05
0.78
5.24
2.66
b)
I:1
I:2
II:1
II:2
II:3
4.55
5.48
III:1
III:2
III:3
III:4
III:5
2.42
0.47
3.35
1.79
-1.04

Figure 6.2

Illustrative family trees of sporadic AOPTD. TDT Z-scores are positioned, in bold, beside each family member examined. Clinically affected individuals have filled icons. Individuals tested for TDT have a central dot; red for abnormal TDT (Z-Score > 2.5) and green for normal TDT (Z-Score < 2.5).

(a) TDTs were examined in five family members including the propositus. The proband (II:1), a 63 year old man had cervical dystonia for 19 years at the time of examination. He had an abnormal TDT Z-Score of 4.33. All four of his asymptomatic children were tested for TDT, two with normal results (Z-Scores 1.05 and 0.78) and two with abnormal results (Z-Scores 5.24 and 2.66).

(b) TDTs were examined in seven family members including the propositus. The proband (II:2), aged 64 years, had oromandibular dystonia and blepharospasm (Meige's syndrome) present for 12 years at the time of testing. She had an abnormal TDT with a Z-score of 5.48. Her older sister (II:1) and one daughter (III:3) were asymptomatic with abnormal Z-scores consistent with autosomal dominant transmission of abnormal TDTs. Four other offspring, all sons, all had normal TDT Z scores.

(TDT: temporal discrimination threshold, AOPTD: adult onset primary torsion dystonia)

Group	Control Subjects	AOPTD Patients			First Degree Relatives				
		All	M	F	All	M	F	Sibs	Off
Number Tested	61	32	12	20	73	34	39	36	36
Number Abnormal TDTs	1	27	10	17	32	12	20	20	11
Percentage Abnormal	2%	84%	83%	85%	44%	35%	51%	56%	31%

Table 6.4: *The number and percentage of abnormal TDT tests in all of the control participants, sporadic AOPTD patients and their first degree relatives by gender and relationship in the study. (TDT: temporal discrimination threshold, AOPTD: adult onset primary torsion dystonia)*

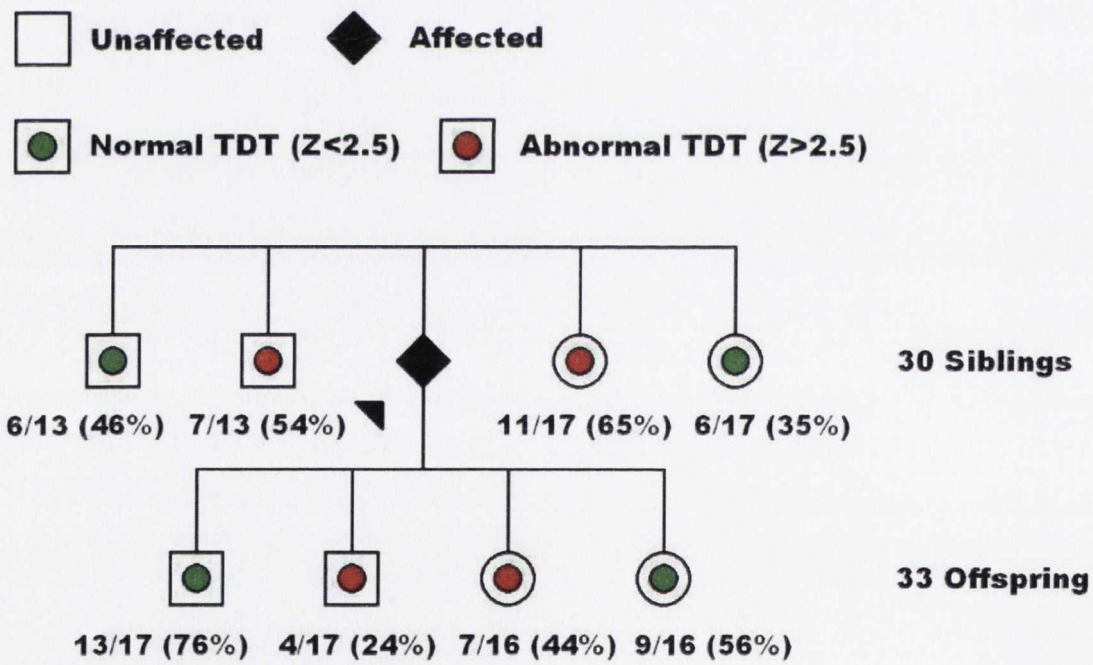


Figure 6.3: *Summary of the frequency of abnormal TDTs in sisters, brothers, daughters and sons of patients with sporadic AOPTD. The proband is the filled diamond icon. Relatives with abnormal TDTs have a red dot and those with normal TDTs have a green dot. In each relationship to the proband, the fractions under an icon represent the ratio of abnormal results to total of that relationship tested, with percentages. (TDT: temporal discrimination threshold, AOPTD: adult onset primary torsion dystonia)*

DISCUSSION

This analysis demonstrates that the TDT is a relatively sensitive measure of abnormal temporal processing in patients with sporadic AOPTD. The frequencies of abnormal TDTs in patients (84%) and their first degree relatives (44%) are consistent with an autosomal dominant endophenotype. When there was relatively complete TDT assessment of the majority of the members of any one family, there was evident autosomal dominant transmission of the abnormal TDT (Fig. 6.2), similar to that found in familial AOPTD (Bradley *et al.*, 2009). One may thus propose that the presence of an abnormal TDT in unaffected relatives of AOPTD patients, both sporadic and familial, is a marker of gene carriage. In support of this I had previously noted an abnormal TDT in an unaffected obligate heterozygote in familial AOPTD (Bradley *et al.*, 2009). Further support for the proposition comes from the evidence of abnormal TDTs in both affected and unaffected gene carriers of *PINK1* (Fiorio *et al.*, 2008) and *DYT1 (Fiorio et al., 2007)*. In order to prove that an abnormal TDT indicates an asymptomatic heterozygote, in the absence of gene identification, it would be necessary to demonstrate that an asymptomatic relative with an abnormal TDT subsequently developed AOPTD. Given the low penetrance of the phenotype, this is an unlikely event.

As discussed in Chapter 4, abnormal TDTs are significantly more frequent than abnormal spatial discrimination thresholds (SDTs) in patients (TDT: 86% vs. SDT: 25%) and first degree relatives (TDT: 44% vs. SDT: 25%) (Bradley *et al.*, 2010). While TDT is a sensitive endophenotype, it is imperfect in that it was abnormal in only 84% of this group of affected patients; in other AOPTD cohorts a higher rate (97%) of abnormal TDTs in cervical dystonia was shown (Bradley *et al.*, 2011). Furthermore, specificity is less than 100%, with an

abnormal combined TDT (Z=2.96) found in one (2%) of the 61 control participants who was 64 years of age. Abnormal TDTs are found in Parkinsonism and when studying TDTs in subjects greater than 60 years, there is the risk of detecting a subclinical basal ganglia disorder, an endophenocopy. In order to ensure 100% specificity, it might be better to confine TDT testing as a marker of gene carriage to individuals less than 50 years of age.

The findings are in keeping with the hypothesis that most, if not all, sporadic AOPTD patients are the only manifesting individuals of an autosomal dominant disorder, because of the low penetrance of the gene or genes causing AOPTD. In this study 22 of the 24 families in which two or more family members were examined by TDT had at least one unaffected family member with an abnormal TDT. The only families in which there was no relative with an abnormal TDT were seven of the nine in which only one first degree relative was examined and two of 15 in which two relatives were tested. As a result of the low penetrance of the gene(s) causing AOPTD, one reason for sporadic AOPTD presentation may be a relatively lower number of first degree relatives in sporadic than in familial AOPTD patients; this needs to be examined further. In addition the ages of siblings and children may be a factor in a disorder which does not become manifest until the fourth decade or later (O'Riordan *et al.*, 2004).

In this study, although not reaching statistical significance, there is evidence of a trend in the difference of the prevalence of abnormal TDTs in unaffected first degree relatives between men and women and also in the frequency of abnormal TDTs with age. Cervical dystonia is more common in women than men (Leube *et al.*, 1997) and this gender effect in the penetrance of the phenotype may also affect the endophenotype, an abnormal TDT. Similarly in relation to age, the mean age of onset of cervical dystonia is 41 years (O'Riordan

et al., 2004) and there was a trend for greater prevalence of abnormal TDTs with age; abnormal TDTs were found in 55% of those over 45 years of age and in 36% of those less than 45 years of age.

Defazio and colleagues have examined the feasibility of using affected sib-pair analysis to search for dystonia genes; because of low penetrance, the resources of a cooperative international study using multiple dystonia cohorts would be needed (Defazio *et al.*, 2006). However such a study using sporadic AOPTD patients and unaffected siblings, less than 50 years of age, with abnormal TDTs would be possible in one centre.

Normal temporal discrimination, as determined by the TDT, is a reflection of effective putaminal processing of sensory stimuli. In one fMRI study subjects were tested with both auditory stimuli, separated by intervals of from one to 20 milliseconds, and tactile stimuli to the left forearm, separated by intervals of from five to 110 milliseconds. When subjects were perceptually certain that the stimuli were either single or double there was activation in the right putamen, at different sites, for auditory and tactile stimuli (Pastor *et al.*, 2008). The authors concluded that the putamen has a central role in the automatic processing of temporally distinct stimuli. Temporal discrimination in the normal putamen occurs with remarkable definition; individuals can process and recognise tactile and visual stimuli separated by less than 1/20 of a second. Such accuracy mediated by sub-cortical – basal ganglia circuits may be of evolutionary significance alerting the individual to environmental stimuli of potential danger (Redgrave *et al.*, 2010). Abnormal temporal discrimination may be a marker of disrupted putaminal function, whether primary or secondary to disordered cortical input (Tamura *et al.*, 2008). Given the presence of abnormal temporal discrimination in Parkinsonism and its improvement with dopamine supplementation (Malapani *et al.*,

1998), a mechanism involving a disorder of dopaminergic transmission seems probable. Abnormal TDTs in AOPTD, DYT1 dystonia, multiple system atrophy, Parkinson's disease and PINK1 parkinsonism may reflect an abnormality in dopamine transmission at differing points of the nigrostriatal-pallidal-thalamic pathway. Abnormal temporal discrimination in non-penetrant AOPTD family members represents a primary subclinical trait which may require other factors, including possibly age and gender, to become clinically manifest as AOPTD.

CONCLUSION

Abnormal TDTs in sporadic AOPTD patients and their unaffected first degree relatives are compatible with an autosomal dominant endophenotype. Most AOPTD patients have a genetic cause with sporadic cases representing the only manifesting carrier in that family. The TDT appears to be a sensitive marker in both manifesting and non-manifesting AOPTD gene carriers and therefore of use in genetic studies of this disorder. The results of the study in this Chapter were published in the peer reviewed journal Brain (Oxford Journals) (Kimmich *et al.*, 2011).

Chapter 7 builds on the accumulating evidence for TDT as an robust endophenotype in AOPTD, presenting a functional MRI study aimed at further validating TDT as an endophenotype and also providing the functional neuro-anatomy of AOPTD.

CHAPTER 7
FUNCTIONAL IMAGING FINDINGS ASSOCIATED WITH TDTS

In Chapter 3 a voxel based morphometry examination demonstrated that bilateral putaminal enlargement was found in unaffected relatives with abnormal TDTs of AOPTD patients when compared to relatives with normal TDTs. Putaminal enlargement is recognized in AOPTD and therefore this disease-associated feature was found in the unaffected relatives with the endophenotype. This both provides validation of the endophenotype and also indicates that putaminal enlargement is probably a primary gene-associated phenomenon rather than a secondary result of abnormal sensori-motor function in these patients. In this Chapter the functional MRI features associated with TDT are examined in AOPTD patients and relatives compared to healthy controls.

BACKGROUND TO THE ANALYSIS

Although adult-onset primary torsion dystonia (AOPTD) is the most common form of dystonia and is inherited in an autosomal dominant fashion with a penetrance as low as 12-15% (Waddy *et al.*, 1991), the paucity of multiplex AOPTD families makes genetic study of the disorder difficult. While it is possible to identify another affected individual in up to 25% of apparently sporadic cases (Leube *et al.*, 1997, Stojanovic *et al.*, 1995), most AOPTD families consist of only two affected individuals and are still small in terms of planning genetic studies. Identification of non-manifesting gene carriers by use of a sensitive endophenotype is one approach to this problem.

Significant sensory processing abnormalities are found in AOPTD patients including abnormalities in spatial discrimination threshold (SDT), temporal discrimination threshold (TDT) and vibration induced illusion of movement (VIIM) (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Frima *et al.*, 2008, Hallett, 1998, Meunier *et al.*, 2001, Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). These sensory abnormalities may be of utility as endophenotypes. In addition, it has been proposed that abnormal sensory processing may play a primary phenomenon in AOPTD, and may play a role in the pathogenesis of AOPTD (Hallett, 1995, Tinazzi *et al.*, 2003).

The Temporal Discrimination Threshold (TDT) is the shortest time interval at which a subject can detect that two stimuli are asynchronous; TDT testing is psychophysiological task that is relatively easy to administer with the advantage of showing significantly less age-dependence (Hoshiyama *et al.*, 2004) than other candidate sensory tests in AOPTD, including such as spatial discrimination thresholds (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). The TDT has been shown to be abnormal in DYT1 patients and non-manifesting DYT1 carriers compared to non-carrier relatives or controls (Fiorio *et al.*, 2007). The TDT has also been shown to be abnormal in patients with writer's cramp (Fiorio *et al.*, 2003), blepharospasm (Fiorio *et al.*, 2008), Parkinson's disease (Artieda *et al.*, 1992, Lee *et al.*, 2005) and multiple system atrophy (Lyoo *et al.*, 2007) and therefore may be a sensitive marker of abnormal sensory integration in the basal ganglia. Further validation of TDT as such a marker would bolster its use in eagerly anticipated genetic studies of AOPTD.

THE BASAL GANGLIA AND TDT

Structure in dystonia has been studied in vivo in humans; a majority of studies report an increase in putaminal volume in AOPTD patients (see more detailed discussion in Chapter 3).

This is not universal, however, with a report of decreased putaminal volume in CD patients (Obermann *et al.*, 2007). In Chapter 3 we discussed increased putaminal volume in unaffected relatives of AOPTD patients with abnormal TDT scores (Bradley *et al.*, 2009). It has been speculated the putaminal enlargement in unaffected relatives could be a protective factor (Draganski *et al.*, 2009).

Functional MRI studies of temporal discrimination in healthy participants have identified the putamen as a structure involved in the early encoding of time intervals of sensory signals (Nenadic *et al.*, 2003, Rao *et al.*, 2001). An early study of temporal discrimination in subjects with focal cerebral lesions found that TDT was increased without evident sensory loss in lesions involving the putamen, among other areas (Lacruz *et al.*, 1991). When two stimuli, auditory or tactile, were given at varying intervals to participants and when they were perceptually certain that the stimuli were either single or double, there was activation in the putamen (Pastor *et al.*, 2008). This activation was contralateral to the stimulus presentation when a stimulus was presented unilaterally. Pastor concluded that the putamen has a central role in the automatic processing of temporally distinct stimuli and acted as a default system for temporal discrimination when there is perceptual certainty. Other regions important for temporal discrimination include the pre-supplementary motor area and the anterior cingulate gyri bilaterally. In comparing auditory discrimination versus controls, Pastor and colleagues demonstrated cortical activation in the right superior gyrus, the right superior, middle and inferior frontal gyri, the right insula in its anterior aspect, and the right anterior cingulate and pre-SMA (Pastor *et al.*, 2004). Subcortical activations were observed in regions of the thalamus, the head of caudate and putamen (Pastor *et al.*, 2006).

FUNCTIONAL MRI AND DYSTONIA

fMRI has been used for some time to investigate movement disorders. For example, a clinical-structural-functional correlation study of patients with pallidal lesions and either hyper- or hypokinetic symptoms revealed that structural changes, with accompanying hypoactivation, in the GPi were associated with dystonic features (due to impaired inhibition of the thalamus) and conversely that lesions in the GPe, with associated hypoactivation, were seen in patients with akinetic-rigid symptoms proposed (due to enhanced thalamic inhibition) (Bucher *et al.*, 1996).

BEFORE FUNCTIONAL MRI

Prior to fMRI, a number of PET studies had been carried out in dystonia patients, with varying results. PET studies using vibration stimuli at the hands dystonia patients to provoke regional blood flow have shown hypoactivation in the contralateral sensorimotor cortex and supplementary motor area, regardless of hand stimulated, in idiopathic focal dystonia (Tempel and Perlmutter, 1990) and writer's cramp (Tempel and Perlmutter, 1993) patients. Additional writer's cramp PET studies showed that writing was associated with hyperactivation of the left primary sensorimotor and premotor cortices, left thalamus, and the right-side predominant cerebellar hyperactivation, correlated with the duration of writing (Odergren *et al.*, 1998), and that writer's cramp patients had decreased sensorimotor cortex activation during sustained contraction and decreased premotor activation during writing along with decreased correlation between premotor cortical regions and putamen, suggesting a network disorder (Ibanez *et al.*, 1999). A further PET study in five idiopathic torsion dystonia patients engaged in hand movement revealed

under-activation in the bilateral sensorimotor cortex as well as the caudal supplementary motor area, posterior cingulate, and mesial parietal cortex while over-activation was seen in the contralateral lateral premotor cortex, rostral supplementary motor area, Brodmann area 8, anterior cingulate, ipsilateral dorsolateral prefrontal cortex, and bilateral lentiform nuclei (Ceballos-Baumann *et al.*, 1995). Another PET study of hand movement in generalised idiopathic dystonia again revealed a decreased blood flow in the contralateral primary sensorimotor cortex and increased regional flow in the left premotor area, supplementary motor area (SMA), anterior cingulate and left dorsolateral prefrontal area as well as in the cerebellum and putamen (Playford *et al.*, 1998). In DYT1 patients, hyperactivation has been reported at rest (increased metabolic activity in the lentiform nuclei, cerebellum, and supplementary motor areas) and during dystonic activity (midbrain, cerebellum, and thalamus) (Eidelberg *et al.*, 1998). The conflicting results of these early studies probably relate to the complex nature of the sensori-motor network disturbances seen in dystonia and the fact that the measurement of increased or decreased function in a region relates not solely to the underlying pathology but to the method of measurement and the task used.

Functional MRI studies of various forms of dystonia have subsequently been reported. Writer's cramp is often chosen due to the localisation of symptoms (hands rather than head or neck) which reduces significant movement artefact in the scanner.

FUNCTIONAL MRI IN FOCAL HAND DYSTONIA

One of the earliest fMRI studies in patients with task-specific hand dystonia (5 guitar players) showed contralateral sensorimotor cortex hyperactivation with bilateral premotor hypoactivation (Pujol *et al.*, 2000). Also in 2000, a study comparing writer's cramp patients to controls during writing showed greater activation of the ipsilateral cerebellum and thalamus in patients along with extension of primary sensorimotor cortex activation caudally and anteriorly towards the premotor association area, suggesting disinhibition and increased basal ganglia output via the thalamus (Preibisch *et al.*, 2001). A further study, again in writer's cramp patients, reported decreased activation in the primary sensorimotor cortex and supplementary motor area during both a relaxation and contraction task implying abnormalities of both excitatory and inhibitory function (Oga *et al.*, 2002). Another fMRI study in 9 idiopathic focal dystonia patients, mostly writer's cramp, during vibriotactile stimulation of digits 2 and 5 revealed no difference in primary sensory cortex activation pattern, but interestingly a difference in the spatial distribution of activation in the primary sensory cortex, with the digit representations having significantly less separation in the dystonic subjects and hypoactivation in the secondary sensory cortex (area 40) (Butterworth *et al.*, 2003). Focusing on the basal ganglia, a further study showed that finger tapping resulted in residual hyperactivation in the caudate, putamen and GP during a subsequent rest phase, not seen in the sensorimotor cortex, and regardless of side tested, indicating measurable intrinsic basal ganglia dysfunction, likely related to impaired inhibition (Blood *et al.*, 2004). Task specific dystonia was again assessed in a study of simple flexion/extension movements of jaw, fingers and toes. This showed hypoactivation in the putamen contralateral to the dystonic limb only, which correlated with disease severity and was

associated with altered somatotopic organisation and decreased distance between body part representations, suggesting a liability to poor selectivity of muscle activation in the putamen (Delmaire *et al.*, 2005). In an important study of 17 writer's cramp patients, activation was compared to controls during performance of a spatial (grating orientation) discrimination task. It was demonstrated that patients had bilateral task-related hyperactivation in the putamen, caudate, GPi and lateral thalamus, and also outside the basal ganglia in the visual cortical areas, left anterior insula and right intraparietal sulcus but not the primary sensorimotor cortex. The basal ganglia findings were more marked in recent-onset disease. They also demonstrated an inverse correlation between dystonia severity and activation in cerebellar and pontine regions (Peller *et al.*, 2006). In a study of 10 WC patients compared to controls, active task (writing with a pencil) was compared to a control task (writing with a finger). This study showed that the patients had relative hyperactivation of the contralateral putamen, primary sensorimotor cortex, supplementary motor cortex, and premotor cortex and of the ipsilateral cerebellar hemisphere compared to controls during the active (pencil writing) task not seen in the finger writing task. It further showed that the active minus control task in patients left residual activation in the contralateral primary sensorimotor cortex, supplementary motor area, premotor area, basal ganglia, bilateral insula, and right cerebellum, which was not seen in controls (where the subtraction of hand movement–related activation left little to no residual activity). They conclude that there is a likely role for both basal ganglia dysfunction and cortical-subcortical circuit dysfunction in writer's cramp (Hu *et al.*, 2006). A further limited fMRI analysis in 1 control, 1 WC patient and 1 WC patient with mirror movements found that the individual with mirror movements had more widespread contralateral and ipsilateral cortical activation than the control or patient with simple writer's cramp, suggesting that this finding

in some writer's cramp patients is related to compensatory overactivity of the cortex or impaired trans-callosal inhibition (Merello *et al.*, 2006). Islam et al examined cortical response to both sensory and motor tasks in 17 writer's cramp patients, and demonstrated that there was consistent hypoactivation of left Brodmann area 4 (primary motor cortex). The results for other areas were heterogeneous, with hypoactivation of the left Brodmann areas 1-3 with right hand motor activity and bilateral hypoactivation of the bilateral somatosensory cortex in single areas with left hand motor activity. They also saw variable hypoactivation in Brodmann areas 6 and 40, concluding that there is baseline underactivity or impaired activation response in a wide range of cortical areas in writer's cramp (Islam *et al.*, 2009). Digit representation in the primary somatosensory cortex of writer's cramp was examined using fMRI and surface mapping techniques in a study by Nelson et al, demonstrating that digit separation in Brodmann area 3 (specifically 3b) showed reduced separation of the representations of digits 1-3 (affected digits) with shift of the representations of unaffected digits 4-5 towards the others, suggesting that overlap, and not merely displacement, are required for symptoms. They also suggest that sensory response in region 3a is deficient in patients, also a possible pathogenic abnormality (Nelson *et al.*, 2009). More recently, a study of simple and complex finger tapping (which did not induce dystonic symptoms) in focal hand dystonia patients revealed there was hypoactivation in multiple regions. In comparing complex to simple tasks, areas that had differential hypoactivation included the supplementary motor area, bilateral lateral premotor areas, posterior parietal regions, left anterior putamen, right anterior GP, right thalamus and bilateral cerebellum. Regions that displayed differential hypoactivation depending on hand used included the primary motor and sensory cortices, the supplementary motor area, the bilateral posterior putamen, bilateral thalamus (VP nuclei),

insula and bilateral cerebellum. Areas relevant to both these "networks" were parts of bilateral primary motor and sensory cortices, premotor, posterior parietal regions, right cingulate, left thalamus (VP nucleus) and bilateral cerebellum . The conclusion was that the patients had significant and widespread hypoactivation, modulated by both task complexity and hand used, in tasks that did not induce dystonia (Wu *et al.*, 2010).

FUNCTIONAL MRI IN OTHER FORMS OF DYSTONIA

Functional MRI has been studied in other forms of dystonia. In an fMRI study of 9 cervical dystonia and 11 blepharospasm patients, Obermann et al reported that during execution of a non-dystonia-related task (forearm grip task) that the dystonia patients had increased activation in the basal ganglia and other subcortical structures; both phenotypes showed activation in the thalamus, caudate, and putamen, while the blepharospasm patients had additional hyperactivation in the globus pallidus (Obermann *et al.*, 2008). The same group published a further study of 17 cervical dystonia patients during a passive sensory task in an unaffected body region and found that patients had relative hyperactivation in the contralateral primary and secondary sensory cortex, the cingulate cortex and cerebellum bilaterally. Interestingly they also reported a positive correlation between SMA activation and botulinum toxin dose and a negative correlation between SMA activation and severity (as measured by the TWSTRS scale), again highlighting the common theme of impaired inhibition (Obermann *et al.*, 2010). In a study of 6 blepharospasm patients, Schmidt et al report that putaminal hyperactivation was consistently seen during blinking in patients compared to controls (Schmidt *et al.*, 2003). A further study in blepharospasm showed that patients had hyperactivation of the anterior visual cortex, anterior cingulate cortex, primary

motor cortex, central thalamus, and superior cerebellum compared to controls (Baker *et al.*, 2003).

Orofacial dystonia has been examined using fMRI. A study of 12 laryngeal dystonia patients during vocalisation showed that patients had hypoactivation of the primary sensorimotor and the premotor and sensory association cortices, not reversed by botulinum toxin treatment (Haslinger *et al.*, 2005). A further study by the same group of both blepharospasm and Meige's (blepharospasm + oromandibular dystonia) patients dystonia showed that both conditions were associated with hyperactivation of the somatosensory cortex and the caudal supplementary motor area bilaterally, while in Meige's syndrome patients only (differentiated by the presence of the oro-mandibular dystonia component), there was hypoactivation of the primary motor and ventral premotor cortical regions. Interestingly, the sensory cortical changes were partly improved by botulinum toxin treatment in the Meige's patients and the motor cortex findings were not (Dresel *et al.*, 2006). Both of these studies employed a "silent event related" approach, meaning that scanning did not take place during performance of the task; this is performed most commonly with auditory pathway or language studies to assist with exclusion of task-related artefact (Amaro *et al.*, 2002).

Musician's dystonia has also been examined. A recent small study of musicians with task-specific dystonia reported hyperactivation in the ipsilateral premotor area for a right hand tapping task and hypoactivation in the left cerebellum for a task involving tapping of both hands. They conclude that compensatory hyperactivation in motor association areas occurs in musician's dystonia (Kadota *et al.*, 2010). In another study of 2 patients with embouchure dystonia, hyperactivation in the somatotopic face representations within the bilateral

primary sensorimotor cortices and the bilateral premotor area was seen during both a dystonia-inducing and a non-dystonia inducing movement (Haslinger *et al.*, 2010).

In an analysis of a single patient with genetically confirmed action-induced myoclonus dystonia, performing a task that induced dystonia (drawing) hyperactivated the thalamus and dentate nucleus compared to a control task that did not induce dystonia (finger snapping) (Nitschke *et al.*, 2006). A further study of 13 clinically affected epsilon sarcoglycan mutation carriers revealed that patients had hyperactivation in the contralateral inferior parietal cortex, ipsilateral premotor and primary somatosensory cortex, and ipsilateral cerebellum compared to controls (Beukers *et al.*, 2010). A further interesting publication by this group examined imprinting; in myoclonus dystonia maternal imprinting has been postulated to exist i.e. only mutant alleles inherited from the father are generally expressed, although manifesting maternal mutant allele offspring are reported (Grabowski *et al.*, 2003). This study of compared 8 paternal mutation carriers to 8 maternally inherited mutation carriers (4 asymptomatic, 4 mildly symptomatic) and controls. They showed that paternally inherited patients had relative hyperactivation in the secondary sensory cortex compared to maternally inherited patients, and that maternally inherited patients had hyperactivation of the supplementary motor area and ipsilateral cerebellum. They also report a number of other non-significant results and conclude that due to the intermediate findings in the maternal allele carriers, that biased allele inactivation, rather than pure imprinting, may occur in this condition, and that this is consistent with the clinical presentation of affected maternal mutation carriers (Beukers *et al.*, 2011).

SUMMARY OF FUNCTIONAL MRI FINDINGS IN DYSTONIA

The results of these studies are relatively heterogeneous, revealing abnormal activation in a variety of areas, predominantly the primary sensorimotor area, basal ganglia, cerebellum, thalamus and association areas. This likely related to the differing patient populations and methods of ascertainment of activity. In particular, tasks that induce dystonia are more likely to reveal pathological hyperactivation (implying impaired inhibition or compensatory overdrive) and in focal hand dystonia, disordered digit region activations are somatotopically disorganised, supporting a liability to poor focusing of fine motor activity. As with VBM studies, the need for carefully planned replication studies in comparable patient groups is important to refine current knowledge.

HYPOTHESIS AND AIMS

Abnormal TDTs in relatives of AOPTD patients represent an endophenotype, and this will be associated with a functional MRI correlate of TDT processing in these relatives. An advantage of examining TDT processing in relatives is that plastic changes due to disease process cannot interfere with relatives' TDT processing (Peterson *et al.*, 2010). Given that putaminal activation correlates with certainty of judgement, one would anticipate that relatives with normal TDTs would have the greater putaminal activation during a temporal discrimination task compared to those with abnormal TDTs. One would also expect differences between groups in areas such as the pre-SMA and cortical regions. The hypotheses were as follows; First, relatives with normal TDTs would have greater putaminal activation during a temporal discrimination task than those relatives with abnormal TDTs. Second, by comparing unaffected relatives with abnormal TDTs to patients with AOPTD

(who also have abnormal TDTs), protective factors would be observed in the unaffected relatives with abnormal TDTs. Third, that the degree of abnormality in temporal discrimination would correlate with brain activation during the temporal discrimination task.

PATIENTS AND METHODS

The research presented in this chapter was carried out in collaboration with Dr. Robert Whelan, Trinity Centre for BioEngineering, Trinity College Dublin and Dr. Okka Kimmich, Department of Neurology, St. Vincent's University Hospital Dublin,

SUBJECTS

Four groups of participants were recruited for the fMRI study. All subjects were right-handed with normal or corrected to normal vision.

Control participants: Twelve healthy control subjects (six women). Their median age was 48.9 years (range 29.9 - 66.9 years). For control participants, exclusion criteria were a history of neurological disease including neuropathy, visual disorder or a history of cerebral, cervical or brachial plexus injury and a family history of dystonia.

Patients with adult onset primary torsion dystonia: Eleven AOPTD patients (six women) with a diagnosis of cervical dystonia, one of whom had also developed spasmodic dysphonia. Their median age was 48.4 years (range 36.9 - 62.9 years). The diagnosis of dystonia was made at a dedicated dystonia clinic by a two neurologists with expertise in movement disorders. All patients were attending a clinic for treatment with botulinum toxin.

Unaffected first-degree relatives: Fourteen first-degree relatives (seven women) with abnormal TDTs of patients with AOPTD. Their median age was 46.9 years (range 27.2- 70.9 years). Ten first-degree relatives (six men) with normal TDTs of patients with AOPTD. Their median age was 40.8 years (range 30.5 – 61.2 years).

None of the 24 unaffected relatives had any symptoms or signs of a movement disorder. Relatives were examined by the research registrars (OK and DB) and had a full medical history and neurological examination including an examination protocol to assess for any evidence of a neurological disorder, in particular a focal dystonia. A video examination of the relatives was not performed.

BEHAVOURAL TDT TESTING

Temporal discrimination threshold testing was carried out as described in detail in Chapter 2 (Bradley *et al.*, 2009). Briefly, testing was carried out in a single session in a sound-proof air-conditioned room. Subjects were tested for two modalities; a visual task (two flashing LED lights) and a tactile task (non-painful electrical stimulation of the index and middle finger). Stimuli were presented at 5-s intervals and the separation between pairs of stimuli was increased in 5-ms steps. The LEDs were positioned 7 degrees into the subject's peripheral field on the side being tested. LEDs were illuminated for 5 ms on each presentation of the stimulus. Electrical stimuli were presented using square-wave stimulators (Lafayette Instruments Europe, LE12 7XT, United Kingdom) and rectangular cloth electrodes (Item # TD-141C1, Discount Disposables Post Office Box 111 St. Albans, Vermont 05478). Stimulus pulse length was set at 5 ms and stimulus current was increased (in 0.1 mA steps) until the subject could reliably detect the stimuli. Each task was performed four times on each side of the body with the median of the 4 trials in each condition (side x task) taken to eliminate

practice effect. The results of the conditions were then averaged to determine the overall TDT in milliseconds. All behavioural TDT results (in milliseconds) were converted to standardised Z-Scores to enable easy comparison of individual results using the formula;

$$\text{Z-Score} = \frac{\text{Actual TDT} - \text{Age-related control mean TDT}}{\text{Age-related control standard deviation}}$$

The control mean and standard deviation used in the formula depend on the age of the subject being calculated (over or under 50 years). Z- scores of equal to or greater than 2.5 were considered abnormal, as previously described.

FUNCTIONAL MRI

MRI DATA ACQUISITION

All study participants were scanned at the Trinity College Institute of Neuroscience, with a 3.0 Tesla Philips Achieva scanner. During presentation of the paradigm, 200 contiguous blood-oxygen-level dependent (BOLD)–sensitive three-dimensional (3D) volume images were acquired in each of three runs. The first four volumes were discarded to avoid T1 equilibrium effects. Functional data were collected using a T2*-weighted echo-planar imaging (EPI) sequence that acquired 39 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE = 30 ms, TR = 2000 ms, FOV 224x224 mm, 64 mm × 64 mm matrix size in Fourier space). A high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230x230 mm, voxel size 0.9×0.9×0.9 mm) was also acquired.

MATERIALS

Tactile stimulation was delivered using MRI-compatible piezoelectric stimulators, custom built by The Magstim Company Limited (www.magstim.com). Visual stimuli were presented via head-mounted mirror displaying a reflection from a back-projection screen. Presentation of stimuli and recording of responses were controlled by software written in Presentation (www.neurobs.com).

FMRI PROTOCOL

The experimental protocol was presented in three runs of approximately seven minutes each. In each run, four block types were presented: two task blocks (one visual and one tactile) and two control blocks (one visual and one tactile) presented in a quasi random order for a total of 20 blocks in each of the three runs. The quasi random restriction was that no more than two task blocks or no more than two control blocks were presented consecutively and also that no more than two blocks of the same modality were presented consecutively. The visual stimuli consisted of yellow circles presented to the right hemifield, displayed for 50 ms on a black background. Tactile stimuli consisted of 50-ms deflections of the piezoelectric stimulator to the right index and middle fingers. Responses were made on a response pad in the subject's left hand.

For task blocks, trials were either simultaneous or sequential: in the former either the circles or tactile stimuli were presented simultaneously and in the latter stimuli were presented at an interval of 100 ms from the offset of the first stimulus to the onset of the second stimulus. Subjects were asked to judge if the stimuli on each trial appeared simultaneously

or separately. For the sequential trials, the order of stimuli appearance was random. The presentation of either sequential or simultaneous trials within a block was selected randomly with the restriction that only 3/4 trials could be either sequential or simultaneous. For control blocks, the upper or lower stimulus (for visual trials) or index and middle fingers (for tactile trials) were presented and subjects were asked to report about the location of the stimulus (top or bottom). In this way, processes such as attention and motor response were common to both task and control blocks. There was a 3-s inter-block interval to allow for task switching. During this interval the instructions for the next block were presented, as was a reminder about the response options. On each trial, a fixation cross (+) appeared in the middle of the screen for 450 ms, followed (on sequential trials) by the first stimulus. The fixation cross remained on the screen for 1,500 ms following offset of the second stimulus. The intertrial interval was randomly selected between 500 and 1500 ms.

STATISTICAL/MRI ANALYSIS

Data were quality controlled by excluding participants who had more than 3 mm movement in any direction in any session. Data pre-processing and analysis were performed with statistical parametric mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK http://www.fil.ion.ucl.ac.uk/spm) running under Matlab7 (Mathworks, Sherborn, MA, USA). Data were realigned to the first functional image acquired. The structural T1 image was segmented using the "Segment" function in SPM8, which utilizes an iterative combination of segmentations and normalizations (Ashburner and Friston, 2005). Next, skullstripping was performed by including only data with values over 0.5 from the segmented grey, white and cerebrospinal fluid images. Coregistration between the

functional and anatomical images was performed using this skull stripped image. Data were then normalized to the Montreal Neurological Institute (MNI) template using the parameter file from the segmentation routine resampled into 2x2x2 mm sized voxels, and were then smoothed using a 6 mm full width half-maximum Gaussian smoothing kernel. Data were high-pass-filtered using a filter width of 128 s.

At the first level (i.e., for each subject) activity on the visual task blocks was contrasted with activity on the visual control blocks and similarly activity on tactile task blocks was contrasted with activity on tactile control blocks. Each event type was used to construct a series of regressors by convolving the event timings with a Fourier set of seven harmonic functions (three sine, three cosine, and one envelope function with a Hanning window of 15 s). This strategy was employed because it allowed us to investigate potentially complex haemodynamic activity without making stringent prior assumptions about its amplitude-time course profile (Balsters and Ramnani, 2008, Josephs and Henson, 1999). The residual effects of head motion were modelled in the analysis by including the six parameters of head motion acquired from the realignment stage of the preprocessing as covariates of no interest in the first level design matrix.

Age and sex were both entered as nuisance covariates in the SPM second level model. Data were compared across the whole brain with a conservative family-wise error (FWE) rate of $p = .05$, with a minimum cluster size of 8 voxels. The SPM Anatomy toolbox was used to extract the per cent signal change (Eickhoff *et al.*, 2005).

RESULTS

BEHAVIOURAL DATA - TDTS

The TDT Z-scores in the four study groups are illustrated in Figure 7.1. For the twelve healthy control participants the mean TDT Z-score was -0.45 (median -0.56) (range -2.21 to 0.72), for the eleven AOPTD patients the mean TDT Z-score was 5.08 (median 4.44) (range 2.65 to 8.69), for the fourteen unaffected first degree relatives with abnormal TDTs, the mean TDT was 4.96 (median 4.47; range 2.88 to 7.14) and for the ten unaffected first degree relatives with normal TDTs, the mean TDT was 1.35 (median 1.63; range -0.82 to 2.2). There was no significant difference in mean ages of the four groups.

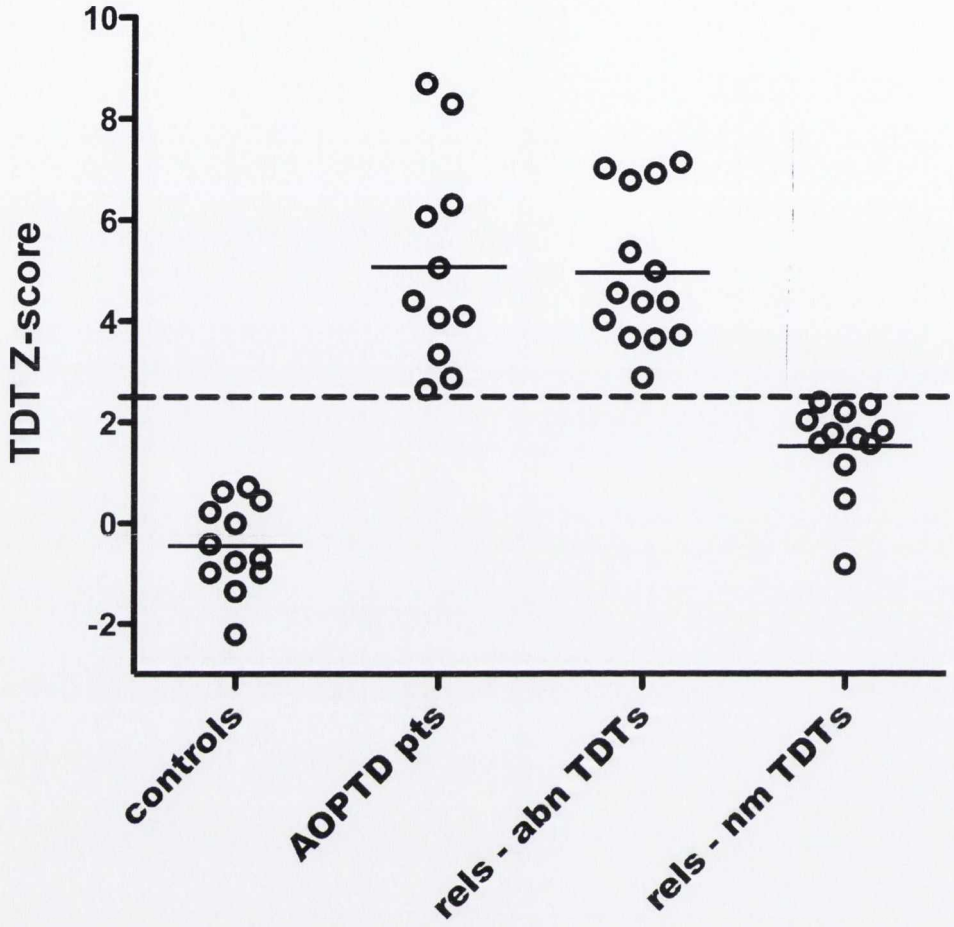


Figure 7.1: *Temporal discrimination threshold test (TDT) results, expressed as Z-scores, in the four study groups (in columns from left to right), 12 healthy control participants, eleven patients with adult onset primary torsion dystonia, 14 first degree relatives with abnormal TDTs and ten first degree relatives with normal TDTs. The broken horizontal line indicates the upper limit of normal for TDT at a Z-score of 2.5.*

FUNCTIONAL MRI ANALYSIS

Accuracy data (mean and standard deviation % correct) were as follows: AOPTD patients, 95.64% (5.17%); relatives with abnormal TDTs, 94.65% (5.84%); relatives with normal TDT 96.39% (4.39%); control participants 96.81% (2.55). There were no significant differences between groups in accuracy ($F = 0.698$, $p = 0.558$). A series of pairwise comparisons contrasting all groups on accuracy scores did not reveal any significant differences, even without correcting for multiple comparisons ($p>0.05$ in all cases). A number of subjects' data did not pass the fMRI quality control procedure (5 patients, 1 abnormal TDT relative, 2 normal TDT relatives, and 2 controls)

RELATIVES WITH ABNORMAL TDTS VS. RELATIVES WITH NORMAL TDTS

The largest difference in activation between relatives with abnormal TDTs versus relatives with normal TDTs was observed in the putamen contralateral to the stimulation (Table 7.1). Relatives with abnormal TDTs also had hypoactivation of the left middle frontal gyrus and the left prefrontal gyrus compared to relatives with normal TDTs. Relatives with abnormal TDTs had greater activation in the right cuneus versus relatives with normal TDTs (Figure 7.2). The BOLD response at the peak voxel in each significant region was then correlated with the TDT Z-score (the Bonferroni correction for multiple comparisons cut-off was set at $p < 0.0125$). Only the BOLD response in the putamen correlated significantly with TDT Z-score. These results are summarized in Table 7.1.

Comparison	Region	BA	L/R	X	Y	Z	Peak Z-Score	Extent	TDT Correlation
Ab. < N.	Putamen	n/a	L	-18	13	-7	7.1	67	-0.603*
Ab. < N.	Mid. Frontal Gyrus	6	L	-30	8	53	6.83	59	-0.415
Ab. < N.	Precentral gyrus	9	L	-34	7	33	5.75	14	-0.327
Ab. > N.	Cuneus	19	R	14	-76	33	5.79	11	0.19

Table 7.1: *Displays the regions that were significantly different between first degree relatives with abnormal temporal discrimination threshold vs. first degree relatives with normal TDT during a temporal discrimination task. Ab: unaffected relatives with abnormal TDTs; N: unaffected relatives with normal TDTs; L: left; R: right; BA: Brodmann area. Coordinates are in Talairach space. *Correlation significant at p<0.0125.*

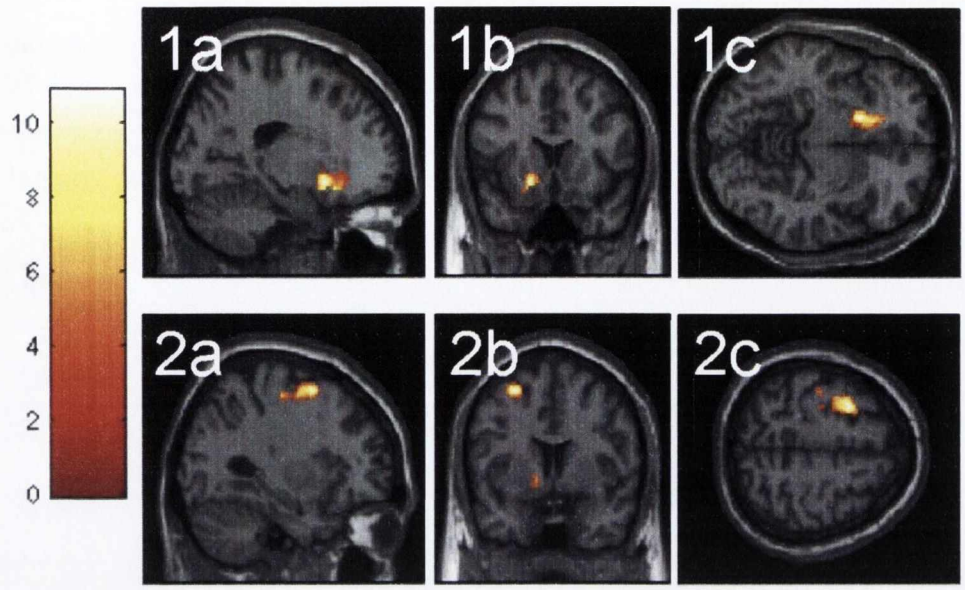


Figure 7.2: *A statistical parametric map of activation for first degree relatives with abnormal TDT vs. first degree relatives with normal TDT. Panel 1 displays voxels in the putaminal region that were significantly hypoactive for relatives with abnormal TDT vs. relatives with normal TDT in a sagittal view (a), a coronal view (b) and an axial view (c). Panel 2 displays voxels in the left frontal lobe that were significantly hypoactive for first degree relatives with abnormal TDT vs. first degree relatives with normal TDT in a sagittal view (a), a coronal view (b) and an axial view (c).The F value is depicted by the colour scale. Voxels that were significant at p<.001 are presented for display purposes.*

RELATIVES WITH ABNORMAL TDTS VERSUS AOPTD PATIENTS.

Table 7.2 summarizes the SPM results comparing relatives with abnormal TDTs versus AOPTD patients. Relatives with abnormal TDTs had hypoactivation, relative to AOPTD patients, in the left pre-supplementary area (pre-SMA), and in two clusters in the left BA7. AOPTD patients, compared to relatives with abnormal TDTs, had hypoactivation in BA 40 and in the right middle frontal gyrus (BA 10) (Figure 7.3). No areas were significantly correlated with TDT Z- score (Bonferroni corrected significance cut-off was p<0.01).

Comparison	Region	BA	L/R	X	Y	Z	Peak z-score	Extent	Correlation with TDT
Ab. < P.	Pre-SMA	6	L	-4	23	38	5.91	17	-0.004
Ab. < P.	Cuneus	7	L	-10	-72	31	5.78	20	0.096
Ab. < P.	Pre-cuneus	7	L	-16	-68	40	5.69	12	-0.002
Ab > P.	Mid. frontal gyrus	10	R	26	48	-7	6.03	9	0.125
Ab. > P.	Sup. parietal lobe	40	L	-34	-52	50	5.23	11	0.381

Table 7.2: *Table 2 displays the regions that were significantly different between first degree relatives with abnormal temporal discrimination threshold vs. AOPTD patients during a temporal discrimination task. Ab: unaffected first relatives with abnormal temporal discrimination thresholds; P: adult onset primary torsion dystonia patients. L: left; R: right; Mid: middle; Sup: superior; BA: Brodmann area; pre-SMA: pre-supplementary motor area. Coordinates are in Talairach space.*

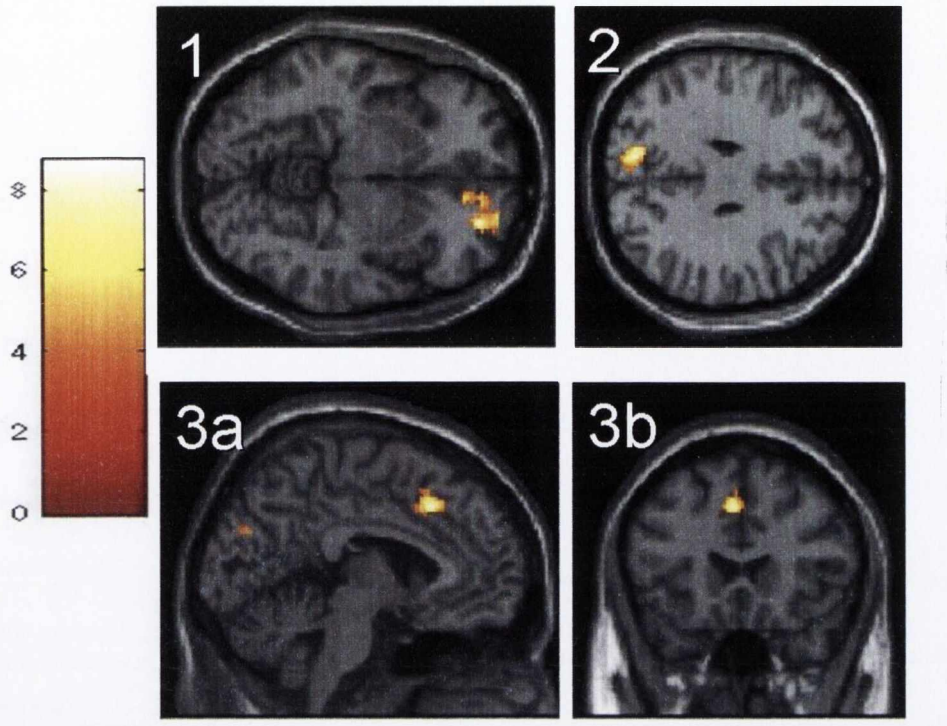


Figure 7.3: *A statistical parametric map of activation for first degree relatives with abnormal TDT vs. patients with adult onset primary torsion dystonia. Panel 1 displays an axial view of the voxels that were significantly hypoactive for patients with adult onset primary torsion dystonia vs. first degree relatives with abnormal TDT in the right middle frontal gyrus. Panel 2 displays an axial view of the voxels in the occipital lobe that were significantly hypoactive in relatives with abnormal TDT vs. patients with adult onset primary torsion dystonia. Panel 3 displays the voxels in the pre-supplementary motor area that were significantly hypoactive for first degree relatives with abnormal TDT vs. patients with adult onset primary torsion dystonia in axial (a) and coronal (b) views. The F value is depicted by the colour scale. Voxels that were significant at p<.001 are presented for display purposes.*

CONTROLS PARTICIPANTS VERSUS AOPTD PATIENTS

Table 7.3 summarizes the SPM results comparing controls and AOPTD patients. Patients had hypoactivation of the dorso-lateral pre-frontal cortex (BA 9) bilaterally, relative to control participants (Figure 7.4). There was no significant correlation between activity in these regions and TDT Z-score.

Comparison	Region	BA	L/R	X	Y	Z	Peak z-score	Extent	Correlation with TDT
P < C	Mid. frontal gyrus	9	R	28	27	26	5.85	9	.014
P < C	Mid. frontal gyrus	9	L	-22	29	26	5.40	9	-.145

Table 3: *Displays the regions that were significantly different between the control and patient groups during a temporal discrimination task. C: healthy controls; P: adult onset primary torsion dystonia patient ; L: left; R: right. Mid: middle; BA: Brodmann area. Coordinates are in Talairach space.*

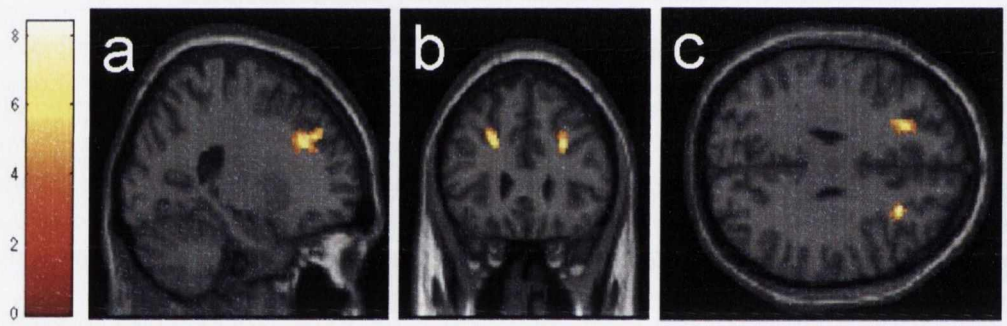


Figure 4: *A statistical parametric map of activation for patients with adult onset primary torsion dystonia vs. healthy controls during temporal discrimination threshold task. Voxels that were significantly hypoactive in the middle frontal gyrus bilaterally for the patients with adult onset primary torsion dystonia vs. healthy controls are displayed in a sagittal view (a), a coronal view (b) and an axial view (c) The F value is depicted by the colour scale. Voxels that were significant at p<.001 are presented for display purposes.*

PUTAMINAL ACTIVATION IN ALL GROUPS.

There was a significant difference in putaminal activation between relatives with abnormal TDTs versus relatives with normal TDTs but no differences in putaminal activation between AOPTD patients and any other group. In order to investigate the putaminal activation further, the signal change at the peak voxel from the abnormal versus normal TDT relatives analysis (i.e., a functionally

defined ROI) comparison was extracted for all four groups (Figure 7.5). The hypothesis was that the analysis between the two relative groups produced a more reliable and unconfounded estimation of the true effect of dystonia gene carriage and choosing the maximal point of difference in that comparison was the most appropriate location use to estimate the true level of putaminal activation relevant to TDT in all groups. It is noted however that this approach carries the potential adverse effects of "double dipping" (the use of the same data set for selection and selective analysis).

The mean putaminal activity of the patient group was lower than either the control group or relatives with normal TDTs. However, the variability of responses was greater in the patient group. Activity of the BOLD response at the putaminal peak voxel was significantly inversely correlated with the TDT Z-score across all subjects (Rho = -0.40, p = 0.005) (Figure 7.6). In AOPTD patients disease duration correlated positively with BOLD activity in the putamen (Rho = 0.772, p = 0.009). Those patients with the longest disease duration had the largest BOLD response.

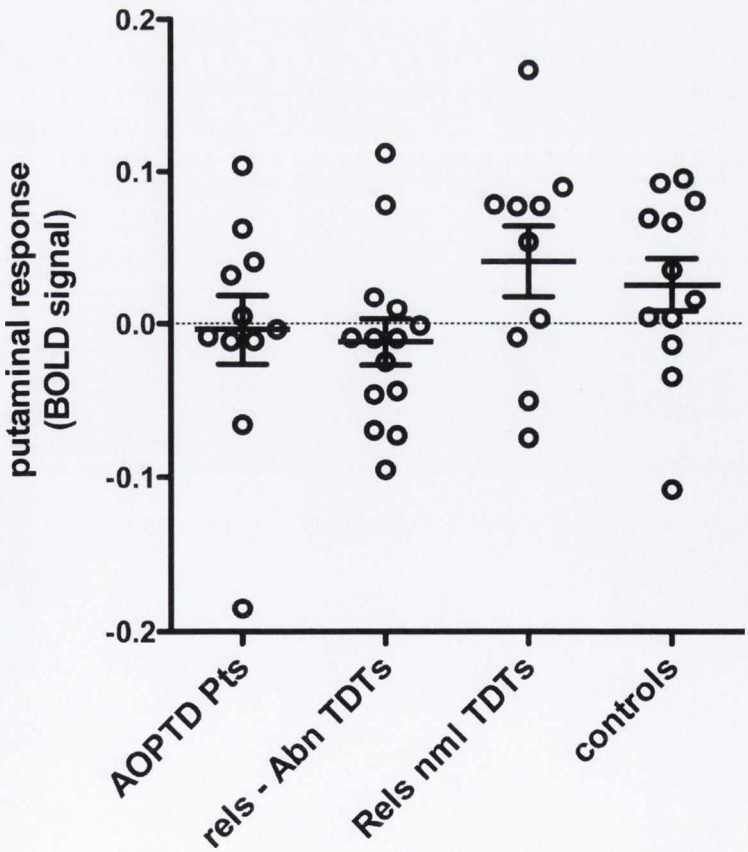


Figure 7.5: *Mean and standard error of the BOLD response for each group in the left putamen (Talairach coordinates = -18, 13, -7) in the four study groups (in columns from left to right), 11 patients with adult onset primary torsion dystonia, 14 first degree relatives with abnormal TDTs, ten first degree relatives with normal TDTs and 12 healthy control participants.*

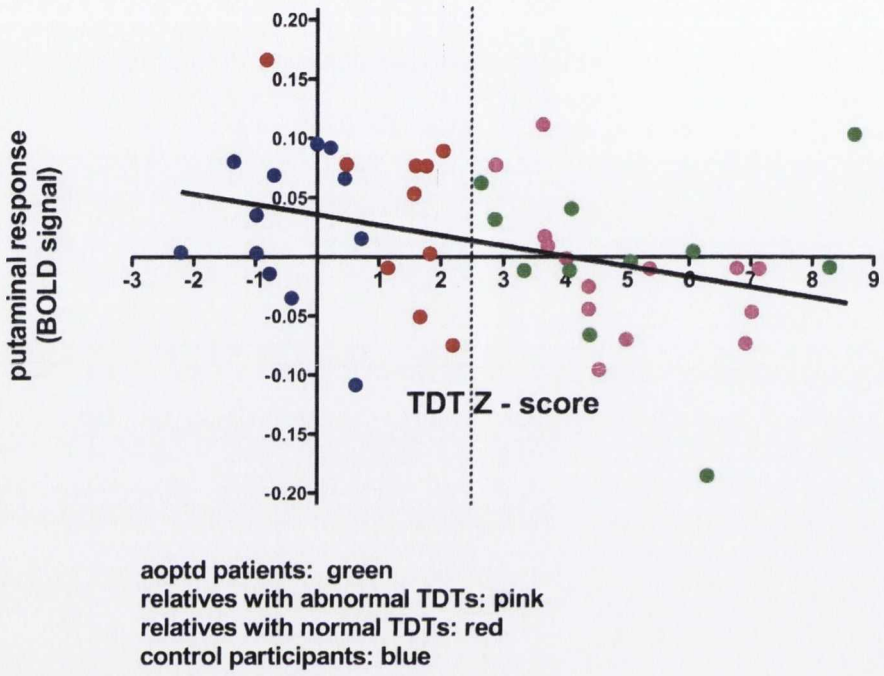


Figure 7.6: *A scatterplot and regression line showing the BOLD response in the left putamen (Talairach coordinates = -18, 13, -7) vs. A z-score of the temporal discrimination threshold for all four groups of participants.*

DISCUSSION

This study demonstrated that first-degree relatives of patients with AOPTD with abnormal TDTs have, by fMRI during a temporal discrimination task, reduced activation in the putamen compared to relatives with normal TDTs. Relatives with abnormal TDTs also had less activity than relatives with normal TDTs in the frontal and precentral gyri (BA 6 and BA 9). Putaminal activation was reduced in AOPTD patients, although not significantly so; there was considerable variability in patients' putaminal responses. For all study subjects the

putamen was the only region that showed a significant negative correlation between TDT Z-scores and activation on fMRI; the greater the abnormality in temporal discrimination, the less the degree of putaminal activation. AOPTD patients had significantly less activity than healthy controls in BA9 bilaterally. Relatives with abnormal TDTs had more activity in the cuneus than relatives with normal TDTs and AOPTD patients had more activity in the cuneus than relatives with abnormal TDT. Accuracy rates did not differ across groups, and therefore the differences in brain activity cannot be attributed to differences in performance.

Patients Compared to Relatives with Abnormal TDTs: Differences between these two groups have the potential to provide insight into disease modifying factors (i.e. protective traits or risk factors for disease expression in those with a dystonia genotype). The results in this analysis revealed that patient with AOPTD had greater activation in three regions (the pre-SMA, cuneus and pre-cuneus) compared to their unaffected relatives with abnormal TDTs, while patients had less activation in two regions (the mid. frontal gyrus and sup. parietal lobe). The pre-cuneus finding is perhaps the most interesting; this area is involved predominantly in episodic memory (Lundstrom *et al.*, 2005, Lundstrom *et al.*, 2003) and visuospatial attention (Cavanna and Trimble, 2006, Kawashima *et al.*, 1995) and has also been proposed as a component of the default mode network (Cavanna, 2007). Given the likelihood that disordered processing of sensory information may play a role in the pathogenesis of dystonic movements, differential activity in this region between manifesting patients and putative non-manifesting carriers may imply that constitutional or acquired dysfunction in this region predisposes to dystonia gene expression. It was also demonstrated that patients had higher pre-SMA activation than controls; this region is known to be recruited specifically in temporal discrimination in healthy control subjects

(Pastor *et al.*, 2004). The region is known to function in temporal processing (Ferrandez *et al.*, 2003, Pouthas *et al.*, 2000, Ramnani and Passingham, 2001, Rao *et al.*, 2001, Schubotz *et al.*, 2000) and likely also has an integrative function (Hernandez *et al.*, 2002, Rizzolatti and Luppino, 2001); again disorder in this region may predispose to the clinical manifestation of dystonia.

PUTAMINAL HYPOACTIVATION AND ABNORMAL TEMPORAL DISCRIMINATION

In Chapter 3 we discussed the fact that relatives with abnormal TDTs had larger putamina, by voxel-based morphometry, than relatives with normal TDTs (Bradley *et al.*, 2009). The finding that relatives with abnormal TDTs, compared to relatives with normal TDTs, have a functional putaminal deficit is consistent with the previous finding of a structural deficit. Although it has been proposed that temporal discrimination may be a cortical function (Tamura *et al.*, 2008), a number of studies now suggest that normal temporal discrimination is primarily a putaminal function. fMRI in normal subjects has shown that the putamen is involved in the early encoding of time intervals, later the insula and dorsolateral prefrontal cortex are activated (Nenadic *et al.*, 2003, Rao *et al.*, 2001). When two stimuli at varying short inter-stimulus intervals are recognised by participants as being distinctly asynchronous or synchronous then putaminal activation occurs. The basal ganglia act as a default system for temporal discrimination unless there is perceptual uncertainty when prefrontal areas become engaged (Pastor *et al.*, 2008). Thus while dysfunction of a number of areas, including cortical regions, may impair a variety of temporal processing tasks, abnormal temporal discrimination is a marker of putaminal dysfunction on dystonia and highly

relevant to the underlying concept of the pathogenesis of dystonia. This concept is supported by the finding of a significant negative correlation between the degree of putaminal activation and worse performance on the TDT task. Reduced D2 receptor availability has been demonstrated in the putamina in both DYT1 and DYT6 manifesting and non-manifesting carriers (Carbon *et al.*, 2009), in DYT1 dystonia (Asanuma *et al.*, 2005) and, more relevant to the present study, in cervical dystonia (Naumann *et al.*, 1998, Perlmutter *et al.*, 1997). In a mouse model of DYT1 dystonia there is evidence that mutant *TorsinA* causes D2R dysfunction (Martella *et al.*, 2009). Mechanisms of long term depression and synaptic depotentiation are impaired in dystonia and the bidirectional nature of synaptic plasticity is impaired in dystonia (Martella *et al.*, 2009, Quartarone and Pisani, 2011). One expression of this disordered plasticity in the putamina of relatives of AOPTD patients may be abnormal temporal processing as evidenced by the TDT.

In AOPTD patients, although they all had abnormal TDTs, as a group there was no evidence of significant putaminal hypo-activation compared to healthy controls. In AOPTD patients disease duration correlated positively with BOLD activity in the putamen (Rho = 0.772, p = 0.009). Patients with the longest disease duration had more putaminal activation than those with shorter disease duration. The explanation for the less marked putaminal hypoactivation in AOPTD patients than in relatives with abnormal TDTS is not clear. All the AOPTD patients were receiving botulinum toxin therapy and although botulinum toxin has no effect on temporal discrimination (Scontrini *et al.*, 2011), a recent study has shown that such treatment reduced the activation of the contralateral putamen in orofacial dystonia during sensory stimulation (Dresel *et al.*, 2011). It is thus possible that the variation in putaminal activation in the AOPTD patient group relates to both disease duration and

treatment effects. Previous fMRI studies of dystonia patients have reported mixed results, with various studies reporting either increases or decrease in basal ganglia activity (Zoons *et al.*, 2011).

TEMPORAL VS. SENSORY ENDOPHENOTYPES

A number of endophenotypes have been proposed in AOPTD; the TDT test has greater sensitivity and specificity than other proposed endophenotypes (Bradley *et al.*, 2010). Abnormal temporal discrimination is found in a number of AOPTD phenotypes including focal hand dystonia (Fiorio *et al.*, 2003, Sanger *et al.*, 2001), blepharospasm (Fiorio *et al.*, 2008, Scontrini *et al.*, 2009), cervical dystonia(Bradley *et al.*, 2010, Bradley *et al.*, 2009) and spasmodic dysphonia (Scontrini *et al.*, 2009). Sensory abnormalities are found in a proportion of AOPTD patients and include impaired spatial discrimination at the fingertips using JVP domes (Molloy *et al.*, 2003, O'Dwyer *et al.*, 2005, Sanger *et al.*, 2001), impaired vibration induced illusion of movement (Frima *et al.*, 2008), altered somatotopy in the cortex (Meunier *et al.*, 2001) and in the putamen (Delmaire *et al.*, 2005). Both the temporal and sensory abnormalities may be manifestations of the impaired inhibition of neuronal activity found in AOPTD (Hallett, 2010). However some evidence suggests that the sensory disorganisation seen in AOPTD may be an adaptive secondary phenomenon rather than an expression of the primary pathogenic process. Prolonged rehabilitation of patients with focal hand dystonia leads to "hyper-normalization" of the cortical representation of the digits of the treated hand, but not of the other hand (Bleton *et al.*, 2011). Botulinum toxin treatment results in a temporary improvement in spatial discrimination in cervical dystonia (Walsh and Hutchinson, 2007), induces changes in the motor cortex somatotopy (Byrnes *et*

al., 1998, Thickbroom *et al.*, 2003) and reduces motor cortex excitability (Gilio *et al.*, 2000). However botulinum toxin has no effect on temporal discrimination (Scontrini *et al.*, 2011). The effect of therapies on cortical plasticity and the lack of effect on temporal discrimination may suggest that the primary disorder in AOPTD is not cortical. Impaired temporal discrimination may be a behavioural marker of the intrinsic pathophysiology of AOPTD given that it is found in unaffected first degree relatives, obligate heterozygotes (Bradley *et al.*, 2009) and its association with structural and functional abnormalities of the putamen.

THE ANATOMY OF TEMPORAL DISCRIMINATION

Normal temporal discrimination can detect changes in the visual and tactile environment within a remarkably defined accuracy of approximately 30 ms and may have evolutionary significance in the detection of visual, auditory or tactile alterations in the environment and of use in detecting prey or avoiding a predator. The anatomical pathway by which salient environmental changes influence motor responses is by a sub-cortical basal ganglia loop (Redgrave *et al.*, 2010). It is thought that a retino-tectal-nigral pathway is used to detect unpredictable, biologically-salient events that interrupt ongoing behavior and attract orienting responses. Visual stimuli reach the substantia nigra pars compacta (SNpc) and the intralaminar nucleus of the thalamus from the superior colliculus. Short- latency visual activation of dopaminergic cells of the SNc occurs via a pathway involving the superior colliculus in the rat (Comoli *et al.*, 2003). Such salient events also access the intralaminar nucleus of the thalamus and thalamic input to the striatum engages cholinergic interneurons in a feed-forward circuit to differentially gate corticostriatal control of

striatopallidal and striatonigral networks (Ding *et al.*, 2010). The effect of a high priority salient signal is to suppress activity in the "no-go" striato-pallidal output and select an appropriate action (Thorn and Graybiel, 2010). The superior colliculus receives input from salient tactile and auditory stimuli as well as visual (Favaro *et al.*, 2011, Redgrave *et al.*, 2010). The temporal discrimination task is a measure of the integrity of this sub-cortical basal ganglia pathway in patients with AOPTD and their relatives with abnormal TDTs, and most evidence points to a disorder in putamen specifically as a cause of abnormal temporal processing in this condition.

CONCLUSION

Temporal discrimination is an index of normal putaminal function in AOPTD. Abnormal temporal discrimination in relatives of patients with AOPTD performing a TDT task is associated with functional hypoactivation of the putamen; this study further validates the TDT as an endophenotype in AOPTD.

CHAPTER 8
SUMMARY AND CONCLUSIONS

OVERALL FINDINGS OF THE THESIS

TDT IN CONTROLS

TDT results in control subjects are relatively resistant to the effect of age (Hoshiyama *et al.*, 2004)– this is in marked contrast to other sensory tests, in particular spatial discrimination testing (O'Dwyer *et al.*, 2005, Walsh *et al.*, 2007). Nonetheless, control subjects are divided into 2 groups to eliminate a small but statistically significant correlation between age and TDT result across all control subjects tested. Control TDT results are closely grouped allowing good discriminatory power and affording TDT higher sensitivity than SDT, for example. This is seen in the significantly greater range of abnormal results (up to Z-Score 20) with TDT testing. This high sensitivity is accompanied by a high specificity – only one of the control group values are outside of the 2.5SD cutoff (possibly indicative of an endophenocopy) and abnormal TDTs in otherwise healthy unaffected relatives of AOPTD patients can more reliably be attributed to basal ganglia dysfunction. These characteristics allow TDT to perform well as a screening tool in the investigation of AOPTD families when assigning gene carriage status.

TDT values in the healthy control subjects are in keeping with other published work; in the series of temporal discrimination thresholds in eighty healthy volunteers by Hoshiyama and

colleagues, the reported mean TDT is 26.1ms at the index finger (Hoshiyama *et al.*, 2004). Tinazzi and colleagues reported a control TDT of 35.48ms in a study of idiopathic dystonia (Tinazzi *et al.*, 1999). The mean TDT in control subjects is lower than the range of 58 to 68 ms reported by Fiorio and colleagues (Fiorio *et al.*, 2007, Fiorio *et al.*, 2003, Fiorio *et al.*, 2008). As discussed in Chapter 2, this difference may be explained by methodological differences.

TDT IN AOPTD PATIENTS AND RELATIVES

Abnormal TDTs (in the larger cohort) are recorded in 83% of AOPTD patients and overall 46% of unaffected first degree relatives with similar rates among sporadic and familial relative. An ideal endophenotype for an autosomal dominant disorder should be abnormal in 100% of affected individuals, 50% of first degree relatives and in no control subjects; the results in Chapter 2 are compatible with this.

IMPORTANCE OF PHENOTYPE AND TASK TYPE

In assessing different AOPTD phenotypes, there similar rates of abnormal TDTs are found in cervical dystonia, writer's cramp, blepharospasm and spasmodic dysphonia patients (Chapter 4). This implies that TDT is a useful tool in the genetic investigation of all AOPTD phenotypes and may be used in families with multiple phenotypes. In addition, it suggests that the basal ganglia dysfunction resulting in abnormal temporal discrimination is a rudimentary and homogenous finding in all AOPTD phenotypes and is consistent with TDT being a state-independent endophenotype.

Comparison of the three task types (visual, tactile and mixed) reveals that the mixed task performs less well (with a sensitivity of 58%) than the other two tasks (which both have a

sensitivity of 83%). This may reflect the fact that there are more potential sources of variability in the use of a multimodal approach to measuring TDT. Omitting this from the testing protocol allows refinement of the testing procedure with shorter testing time.

The concordance between the three individual TDT tasks is lower in AOPTD patients (76%) and unaffected relatives (77%) than in control subjects, who had 100% concordance. There is a higher frequency of abnormal results using the combined TDT compared to any individual task. Using the combined TDT, abnormal status can be assigned in some subjects with abnormalities in two TDT tasks when the third TDT task was normal. For example, 52% of the group of first degree relatives have abnormal status using combined TDT while the proportions who have an abnormal visual and tactile TDT are 50% and 45% respectively.

STRUCTURAL MRI – VOXEL BASED MORPHOMETRY

The structural MRI findings help to validate TDT as an endophenotype. The study demonstrated an AOPTD-associated phenomenon (bilateral putaminal enlargement) in unaffected relatives with the endophenotype compared to those without. Increased putaminal volume is a consistent finding associated with manifesting AOPTD patients including those with idiopathic blepharospasm (Etgen *et al.*, 2006), focal hand dystonia and cranial dystonia (Black *et al.*, 1998). A recent paper suggested putaminal enlargement is a primary feature of adult onset dystonia and provided evidence that, in DYT1 dystonia, it is due to both gene and disease manifestation effects (Draganski *et al.*, 2009). An fMRI study of temporal processing of an auditory task shows that initial activation occurs in the striatum, particularly the putamen, followed later by more diffuse activation (Rao *et al.*, 2001), supporting the hypothesis that the basal ganglia, and possibly dopaminergic pathways in particular (Malapani *et al.*, 1998), act as a basic time processor in the CNS.

Further fMRI studies have confirmed the central role of the putamen in temporal processing and have found activation lateralised to the right hand side (Nenadic *et al.*, 2003, Pastor *et al.*, 2008). Interestingly, Pastor and colleagues also demonstrated that activation in the putamen decreases with perceptual difficulty suggesting it is primarily involved in automatic perception of time (Pastor *et al.*, 2008). It is reasonable, therefore, to postulate that a disorder of sensory integration in the basal ganglia involving the putamen in particular is the patho-physiological basis of abnormal temporal discrimination AOPTD gene carriers.

There are many outstanding questions relating to the multitude of abnormal experimental findings in AOPTD and whether these represent primary phenomena or secondary features of disease manifestation (Breakefield *et al.*, 2008). The novel demonstration of increased putaminal volume in asymptomatic relatives with abnormal temporal processing is helpful in this regard. This finding suggests that putaminal enlargement is a primary phenomenon in AOPTD gene carriers and is associated with abnormal temporal processing in contrast to the suggestion that putaminal enlargement in AOPTD is secondary to abnormal dystonic motor activity (Etgen *et al.*, 2006).

COMPARISON TO OTHER ENDOPHENOTYPES

In AOPTD patients a remarkable level of discordance (67%) is seen between the SDT and TDT test results. In the unaffected first degree relatives, although both tests are abnormal in a significant proportion (SDT 50%, TDT 41%), there is again a notable discordance of 62%. Clearly one of these two potential endophenotypes is less reliable than the other. The frequencies of abnormalities in AOPTD patients (SDT 21%, TDT 83%) indicate that TDT is a more sensitive marker of abnormal sensory processing in AOPTD. Moreover, in control subjects the distribution of TDT results was narrower (range -2.21 SD to +1.79 SD) than the

SDT control range (range -2.06 SD to +2.63 SD) (Walsh and Hutchinson, 2007) suggesting greater confidence that an abnormal result is indicative of abnormal central sensory processing. Furthermore the range of abnormal Z scores for the TDT is much greater than that of the SDT.

The SDT is relatively sensitive to age related changes in the peripheral nervous system; a number of discordant results may thus be due to the lower specificity of SDT testing. There is marked increase in the sensory threshold with age which reflects the natural effect of age on the peripheral nervous system. This age effect renders it impossible to determine with accuracy the upper limit of normal of the SDT over the age of 65 and probably limits sensitivity of the test over the age of 50. This variation in the SDT sensitivity with age might partly (but not completely) explain why AOPTD patients (who had a mean age of 52 yrs) had fewer abnormal SDT results than their unaffected first degree relatives (mean age 42 yrs).

SDT has more potential for error due to the variability in stimuli presented to subjects using manually applied JVP domes in comparison to the electronically-determined electrical stimuli in the TDT testing procedure. The basal ganglia (Rao *et al.*, 2001), and dopaminergic pathways in particular(Malapani *et al.*, 1998), play a particular role in timekeeping in the CNS. Thus the TDT may be a more sensitive measure of the postulated dopaminergic dysfunction in AOPTD patients (Carbon *et al.*, 2009).

Candidate AOPTD endophenotypes include SDT, TDT, VIIM, PET and TMS. The relative advantages and disadvantages of these techniques as potential endophenotypes have been examined in a number of patient populations.

WHAT TDT TELLS US ABOUT SPORADIC AOPTD

In Chapter 6 we learned that, TDTs are present and transmitted in a manner consistent with a sensitive and specific autosomal dominant endophenotype. Abnormal TDTs were present in 84% of patients and 44% of first degree relatives. There was no effect of parent on transmission but TDTs were more often abnormal in siblings compared to offspring (60% and 33% respectively). There was a trend towards higher prevalence of abnormal TDTs in female relatives, a finding that would complement the observation that cervical dystonia is more prevalent in females for example (Leube *et al.*, 1997). The findings in this analysis support the hypothesis that sporadic AOPTD patients do indeed reflect the poor penetrance of dystonia genes, and represent the only manifesting member in their family. There is scope to conduct a genetic study in sporadic patients and relatives with normal or abnormal TDTs using various techniques (Defazio *et al.*, 2006).

FUNCTIONAL MRI AND TEMPORAL DISCRIMINATION THRESHOLDS

The analysis in Chapter 7 provides further convincing evidence for TDTs as a robust AOPTD endophenotype. The results indicate, as hypothesised, that unaffected relatives with abnormal TDTs have less putaminal activation during temporal discrimination tasks than their counterparts with normal TDTs , most probably related to their reduced certainty performing the task. As such, the abnormal TDT is validated as a marker of impaired temporal processing in these individuals. This reduction in putaminal activation correlated with the magnitude of TDT abnormalities on behavioural testing. The finding that putaminal hypoactivation in the AOPTD patient group was not a statistically significant finding probably reflects the wide variation in activation seen in the specific subjects and may relate to secondary adaptive changes (AOPTD patients with longer duration of illness had less

putaminal hypoactivation). Hypoactivation in Brodmann Area 9 in patients and relatives with abnormal TDTs was also seen, along with hypoactivation of Brodmann Area 6 in the relatives with abnormal TDTs and there was a graduated hyperactivation of the cuneus with greater activation in relatives with abnormal TDTs compared to their normal TDT counterparts, and higher activation again in the patients. The question of whether putaminal hypoactivation during a temporal discrimination task reflects an essential defect of putaminal function in gene carriers for AOPTD; one could argue that hypoactivation of that region simply reflects poor temporal processing ability from any cause. The importance of the putamen is clear in many other dystonic disorders and there are structural abnormalities (bilateral putaminal enlargement) in AOPTD patients (Black *et al.*, 1998, Bradley *et al.*, 2009, Etgen *et al.*, 2006).

LIMITATIONS OF TDT AS AN ENDOPHENOTYPE

TDT testing appears capable of assigning status to individuals. However, TDT is not without limitations; false negative and false positive results occur. In AOPTD patients tested to date, 8/81 (10%) have normal TDTs. Furthermore, 1/61 control subjects (1%) had an abnormal TDT result (Z-Score 2.9) and as part of an collaborative genetic analysis, it was found that removing one unaffected relative with an abnormal TDT (Z-Score 6.6) from a linkage analysis resulted in a significant increase in the LOD score to greater than +3.0 (unpublished results). A false positive TDT was found in the control group in a study of TDT in PINK1; one of the control subjects had a TDT greater than the chosen cut off for normal of two standard deviations above the control mean (Fiorio *et al.*, 2008). Overall however, the number of inappropriate results seems to be low. It is of critical importance that an endophenotype misclassifies as few individuals as possible since such incorrect assignments in a linkage

analysis can negatively affect the outcome. One example of this relates to a Swiss family with dopa-responsive dystonia originally incorrectly assigned to DYT14 (Wider *et al.*, 2008). In addition, while TDT appears to be relatively sensitive in detecting subclinical basal ganglia dysfunction, it is not specific to AOPTD because abnormal TDTs are seen in other basal ganglia disorders. A number of proposed AOPTD endophenotypes do not reliably dichotomise unaffected relatives to allow assignment of probable gene carriage.

CONCLUSIONS

Temporal Discrimination Threshold testing fulfils many of the criteria for a useful endophenotype in AOPTD. Frequencies in AOPTD patients and relatives, transmission patterns in familial AOPTD pedigrees and the finding of a disease-associated pathological finding (bilateral putaminal enlargement) in endophenotype carriers all support its role as an AOPTD endophenotype. The findings in sporadic AOPTD patients and relatives support the hypothesis that all of these cases are genetically determined and the apparent frequency of sporadic cases relates to poor penetrance. The visual and mixed TDT tasks have a higher sensitivity than the mixed task. TDT performs similarly in different AOPTD phenotypes. VBM findings suggest that putaminal enlargement in AOPTD is a primary phenomenon. fMRI findings further validate the endophenotype. Comparison to other candidate endophenotypes, including SDT, reveals that TDT is a more sensitive and reliable marker of subclinical disease than alternative measures. TDT is likely to be a useful tool in AOPTD genetic research.

GLOSSARY AND ABBREVIATIONS

A2A A type of adenosine (a nucleoside containing adenine as its base) receptor.

ACh Acetylcholine, a neurotransmitter.

AOPTD Adult-Onset Primary Torsion Dystoina; the commonest form of dystonia characterised by onset over age 26 and focal or segmental distribution. Postulated to be autosomal dominant with markedly reduced penetrance.

Autosomal Dominant A pattern of genetic inheritance in which an affected individual has one copy of a mutant gene and one normal gene on a pair of chromosomes. In autosomal dominant conditions, the presence of only one copy of the mutant gene is required for disease occurrence (In contrast to autosomal recessive diseases which require that the individual have two copies of the mutant gene). Individuals with autosomal dominant diseases have a 50% chance of passing the mutant gene to each child. In some conditions, even when the gene is present, the disease may not manifest ("non-penetrant gene carrier").

Basal Ganglia A collection of subcortical (deep brain)l structures involved in movement selection and focusing of motor activity; involved in the integration of central and peripheral sensory and motor information to co-ordinate movement.

BEB Blepharospasm; A form of adult-onset primary torsion dystonia (AOPTD) localised to the peri-ocular muscles (also abbreviated to BLEPH).

Broadmann (Areas) Nomenclature used to denote regions in the functional organisation of the cerebral cortex.

BTX Botulinum Toxin; six serotypes exist (A-F).

Carrier Individual that carries the gene for a disease; for autosomal dominant disorders often used to indicate in individual that has the gene but does not manifest the physical expression (phenotype) of the disease.

CD Cervical Dystonia; A form of adult-onset primary torsion dystonia (AOPTD) localised to the neck muscles.

CNS Central nervous system.

CSF Cerebrospinal fluid.

D1; D2 Types of dopamine receptor.

DBS Deep brain stimulation.

DRD Dopa-responsive dystonia.

DRPLA Dentatorubral-pallidolysian atrophy.

Dystonia A common hyperkinetic movement disorder, generally defined as a "syndrome of sustained muscle contractions, frequently causing twisting and repetitive movements, or abnormal postures" (Fahn, 1988, Fahn *et al.*, 1987).

DYT... The prefix applied when naming newly discovered genetic forms of primary dystonia.

DYT1 The prototypical primary genetic dystonia; an autosomal dominant young onset, limb onset dystonia due to e mutation in the gene encoding *Torsin A* on chromosome 9q34.

EMG Electromyography, a technique that measures electrical muscle activity through a needle electrode.

Endophenotype A marker of subclinical gene carriage; a biomarker that fulfils specific criteria and indicates that an individual carries the gene for a disorder even though they are not manifesting the full expected disease phenotype (i.e. they are non-penetrant gene carriers)

Extrafusal Fibres The fibres found outside the muscle spindle in a muscle – i.e. the majority of the fibres in a muscle.

FDR False discovery rate; In structural and functional imaging studies, an option for setting the level of statistical significance; means the proportion of significant differences that are likely to be false positive results (e.g. FDR set at 5% suggests that amongst all of the statistically significantly different voxels, up to 5% are likely to be false positive results).

Familial Disorder occurring where a positive family history can be idenfitied.

FHD Focal hand dystonia; A form of adult-onset primary torsion dystonia (AOPTD) localised to the hand and occurring only during a specific task, often writing, when it is also called writer's cramp (WC).

fMRI Functional magnetic resonance imaging, a technique whereby differences in the amount of activity (function) in the brain, or specific regions in the brain, can be compared between two groups of people.

FWE Family-wise error; In structural and functional imaging studies, an option for setting the level of statistical significance by correcting for multiple comparisons; for example FWE set at 5% means the that the likelihood of any individual statistically significant voxel difference being a false positive is 5%.

GABA Gamma-aminobuytric acid, an inhibitory neurotransmitter

Genotype The presence or not of the gene for a disease.

GP Globus pallidus, also called the pallidum. Has 2 components, the gobus pallidus pars interna (GPi) (this is the same as the medial globus pallidus (MGP)) and the globus pallidus pars externa (this is the same as the lateral Globus Pallidus (LGP)).

GPe Globus pallidus pars externa. ***This is the same as the Lateral Globus Pallidus (LGP).***

GPi Globus pallidus pars interna. ***This is the same as the medial globus pallidus (MGP).***

Intrafusal Fibres The muscle fibres found inside the muscle spindle in a muscle.

LED Light-emitting diode

LGP Lateral globus pallidus. ***This is the same as the globus pallidus pars externa (GPe).***

Linkage In genetics, the tendency for genes to be inherited together because of they are located close to each other on the same chromosome.

Linkage Study Type of genetic study where regions of interest to search for unknown genes are identified at particular locations (loci) because nearby identifiable markers are found to be more common in patients with the condition: the hypothesis is that the known marker and the unknown gene are linked.

Locus The point a gene occupies on a chromosome (pleural = loci)

LOD Score Logarithm of odds score; in genetic linkage studies, this provides a statistical estimate of the likelihood that two loci (genes) lie near each other on a chromosome and therefore whether they are linked (inherited together as a set).

M-Response Waveform obtained by recording over a muscle belly while the nerve supplying that muscle is stimulated electrically; an estimation of the total

motor neuron (extrafusal) transmission to that muscle. Same as the motor evoked potential (MEP).

Meige Syndrome The combination of oromandibular dystonia (OMD) and blepharospasm (BEB); A form of adult-onset primary torsion dystonia (AOPTD).

MELAS Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes.

MEP Motor evoked potential, the vaveform obtained by recording over a muscle belly while the nerve supplying that muscle is stimulated electrically; an estimation of the total motor neuron (extrafusal) transmission to that muscle. Same as the M-Response.

MERRF Myoclonic Epilepsy with ragged red fibres.

MGP Medial globus pallidus. ***This is the same as the globus pallidus pars intera (GPi).***

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahytropyridine

MRI Magnetic resonance imaging

Multiplex A family (pedigree) containing 3 or more individuals with a genetic disorder under investigation.

Muscle Spindle A specialised sensory organ found in skeletal (voluntary) muscle and innervated by gamma nerve fibres. This organ monitors stretch in the muscle belly, required in managing muscle tone and position, and is responsible for the tendon reflex. The muscle fibres found in the muscle spindle are referred to as "intrafusal" fibres.

Musicians Dystonia A form of adult-onset primary torsion dystonia (AOPTD) localised to the instrument-playing body part of a musician.

OMD Oro-mandibular dystonia; A form of adult-onset primary torsion dystonia (AOPTD) localised to the jaw and mouth muscles.

Pallidum Another term for the globus pallidus

PANK Pantothenate kinase-associated neurodegeneration

PAS Paired associative stimulation, a transcranial magnetic stimulation (TMS) technique used to examine plasticity.

Pedigree An illustrated family tree containing individuals with a genetic disorder under investigation, of informally a family carrying a genetic disorder.

Penetrance The expression, or frequency of expression, of the expected physical manifestation of a condition (phenotype) in those with the gene for that condition (genotype); the likelihood that presence of the gene will actually result in manifestation of the disease.

PET Positron Emission Tomography, a type of functional imaging technique in which differences in the amount of activity (function) in the brain, or specific regions in the brain, can be compared between two groups of people.

PINK1 "PTEN-induced kinase 1". A gene that causes an autosomal recessive form of inherited Parkinson's Disease.

PKC Paroxysmal kinesogenic choreo-athetosis

Plasticity A feature of the nervous system whereby function (effectiveness of transmission) of established neural circuits can change over time.

PNKD Paroxysmal non-kinesogenic dyskinesia

PPN Pedunculo-pontine nucleus.

Proband The index (first identified) individual in a family (pedigree) witht eh disorder under investigation.

Propositus The relation immediately above an individual of interest in a genetic pedigree (i.e. the parent that transmitted a genetic disorder to an affected individual of interest)

rTMS Repetitive transcranial magnetic stimulation (TMS), a technique used to examine plasticity.

SD Spasmodic dysphonia, a laryngeal dystonia; A form of adult-onset primary torsion dystonia (AOPTD) localised to the laryngeal muscles and affecting voice.

SDT Spatial discrimination threshold, a potential AOPTD endophenotype.

S1 The primary sensory cortex.

SMA Supplementary motor area.

SN Substantia nigra, a component of the basal ganglia circuit, comprising two parts – the substantia nigra, pars compacta (SNc) and the substantia nigra pars reticulate (SNr).

SNAP-25 A synaptosomal protein weighing 25kD; the target of botulinum toxin types A, C and E.

SNARE Soluable NSF [N ethylmaleimide-sensitive fusion] attachment protein receptor – a protein involved in transporting vesicles containing acteylcholine to the neuron curface to enable activation of muscle - acted on by botilinum toxin.

SNc Substantia nigra pars compacta, a component of the sunstantia nigra.

SNr Substantia nigra pars reticulata, a component of the substantia nigra.

SPM5 Statistical parametric mapping software, version 5; computer software used in the analysis of structural and functional imaging studies.

Sporadic Disease occurring without any identifiable family history.

State-Independent A characteristic of endophenotypes whereby the marker is unchanged by the manifestation or not of the disease phenotype or by treatment of the disease.

STN Subthalamic nucleus, a component of the basal ganglia circuit.

TDT Temporal Discrimination Threshold; the shortest time interval at which 2 stimuli are determined to be asynchronous.

THAP1 The gene encoding the "thanatos-associated protein domain-containing apoptosis-associated protein 1", the genetic cause of DYT6 dystonia.

TMS Transcranial magnetic stimulation, a technique used to examine neural excitability and plasticity.

Torticollis Turning or twisting movements, typically referring to these features in the neck muscles of patients with cervical movements

TWSTRS Toronto western spasmodic torticollis rating scale; a clinical scale used to record the clinical severity of the cervical dystonia in patients with the condition.

VAMP Vesicle-associated membrane protein – the target of botulinum toxin types B, D and F.

VBM A structural MRI technique where microscopic differences in the size (volume) of parts of the brain between two groups of people can be detected.

VIIM Vibration-induced illusion of movement, a potential AOPTD endophenotype; the illusion of movement of a limb induced by vibratory stimulation to the muscle (spindle).

Voxel In structural and functional imaging analysis, uniform cubic spaces that the images are divided into for analysis; size varies by study and imaging type.

WC Writer's Cramp; A form of adult-onset primary torsion dystonia (AOPTD) localised to the hand and occurring predominantly or only during writing – usually analogous to focal hand dystonia (FHD)

Z-Score A standardised score that can be applied to an individual participant's test result (e.g. their TDT or SDT result) based on the mean and standard deviation of the control group relevant to that individual's age; this facilitates the analysis of test results across age groups. The Z-Score is the number of standard deviations that that individual's result lies above (+) or below (-) their respective control group mean. Typically a Z-Score of $\geq +2.5$ is considered abnormal.

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