The Endophenotype and the Phenotype: Temporal Discrimination and Adult-Onset Dystonia

Michael Hutchinson, FRCP,1,2* Okka Kimmich, MB,1,2 Anna Molloy, MRCPI,1,2 Robert Whelan, PhD,3,4 Fiona Molloy, FRCP,5 Tim Lynch, FRCP,6 Daniel G. Healy, MB, PhD,7 Cathal Walsh, PhD,8 Mark J. Edwards, PhD,9 Laurie Ozelius, PhD,10 Richard B. Reilly, PhD,3 and Seán O’Riordan, FRCP1,2

1Department of Neurology, St. Vincent’s University Hospital, Dublin, Ireland
2University College Dublin, Dublin, Ireland
3Department of Psychiatry, University of Vermont, Burlington, Vermont, USA
4Department of Neurophysiology, Beaumont Hospital, Dublin, Ireland
5Department of Neurology, Mater Misericordiae University Hospital, Dublin, Ireland
6Department of Neurology, Beaumont Hospital, Dublin, Ireland
7Department of Statistics, Trinity College, Dublin, Ireland
8Department of Genetics and Genomic Sciences and Neurology, Ichan School of Medicine at Mount Sinai, New York, New York, USA

ABSTRACT: The pathogenesis and the genetic basis of adult-onset primary torsion dystonia remain poorly understood. Because of markedly reduced penetrance in this disorder, a number of endophenotypes have been proposed; many of these may be epiphenomena secondary to disease manifestation. Medial endophenotypes represent gene expression; the study of trait (endophenotypic) rather than state (phenotypic) characteristics avoids the misattribution of secondary adaptive cerebral changes to pathogenesis. We argue that abnormal temporal discrimination is a mediational endophenotype; its use facilitates examination of the effects of age, gender, and environment on disease penetrance in adult-onset dystonia. Using abnormal temporal discrimination in unaffected first-degree relatives as a marker for gene mutation carriage may inform exome sequencing techniques in families with few affected individuals. We further hypothesize that abnormal temporal discrimination reflects dysfunction in an evolutionarily conserved subcortical-basal ganglia circuit for the detection of salient novel environmental change. The mechanisms of dysfunction in this pathway should be a focus for future research in the pathogenesis of adult-onset primary torsion dystonia. © 2013 International Parkinson and Movement Disorder Society

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Adult-Onset Primary Torsion Dystonia: The Problem

Adult-onset primary torsion dystonia (AOPTD) is the most common form of dystonia, expressed as various phenotypes thought to result from a combination of shared common susceptibility genes with additional individual genetic and environmental risk factors. Heterogeneity of the phenotype among affected individuals in multiplex families supports this assumption.1,2 In families with 2 members affected by AOPTD, proband-relative pairs had different phenotypes in 46% of pairs.3 There is also evidence that the phenotype is determined by the age of the patient at disease penetrance.2 Segregation analyses suggest an
autosomal dominant inheritance with markedly reduced penetrance of 10% to 15%. Therefore, most AOPTD cases appear to be sporadic and genes affecting only a few families have been identified to date.

Endophenotypes are subclinical quantitative markers of gene expression and their use may help to elucidate the genetic basis and pathogenesis of poorly penetrant disorders. Several putative endophenotypes in AOPTD have been proposed. In this work we argue that abnormal temporal discrimination threshold (TDT) is a valid endophenotype in AOPTD and indicates a distinct pathogenetic mechanism which involves a subcortical–basal ganglia circuit. The main input to this circuit, the superior colliculus, responds to multiple sensory stimuli for the detection of biologically salient environmental events. We also argue that reported cortical abnormalities in AOPTD probably represent secondary phenomena (secondary endophenotypes) rather than primary pathogenetic mechanisms of AOPTD.

**Endophenotypes: Mediation and Others**

Although applied mainly in psychiatric research, the endophenotype concept is relevant to poorly penetrant forms of dystonia. The salient characteristics of an ideal endophenotype have been defined by various authors.

The mediational endophenotype model implies that the endophenotype and the disease are both caused by a genetic disorder and that the pathway from gene to disease passes through the endophenotype; one cannot acquire the disease without first having the endophenotype. A mediational endophenotype reflects disease susceptibility, is not altered by disease severity, is closer to genetic mechanisms of expression and is more penetrant than the phenotype. Mediational endophenotypes by necessity in an autosomal dominant disorder such as AOPTD, should be found, depending on penetrance, in up to 50% of unaffected first-degree relatives. Very few of the postulated endophenotypes in AOPTD fulfill this criterion.

**Some Endophenotypes Are Epiphenomena**

An endophenotype may be an epiphenomenon if both the disease and the endophenotype are caused by a genetic abnormality but there is no other relationship between them (the liability index model). The more common epiphenomenon occurs when the endophenotype results from disease manifestation with adaptive, compensatory, secondary cerebral changes; this is a secondary endophenotype (Fig. 1). Mediational endophenotypes are found in non-manifesting gene carriers and represent a trait, whereas secondary endophenotypes are found only in the disease state. There has been a tendency in the last 10 years in the movement disorder literature to use the term endophenotype to describe findings on investigation that associate with the disease state and which are presumed to be pathogenetic. We argue that these are epiphenomena arising from either of these 2 mechanisms (liability index or secondary).

**Trait Versus State in AOPTD**

Secondary endophenotypes may include cortical changes detected by neurophysiological, imaging, and biophysical tests in various AOPTD phenotypes. For example, impaired intracortical inhibition and plasticity have been found in focal hand dystonia and cervical dystonia, but have not been shown to be abnormal in unaffected first-degree relatives. Treatment with botulinum toxin, in patients with upper limb dystonia, temporarily alters cortical excitability and restores intracortical inhibition by decreasing muscle spindle input 1 month after injection; this, however, was not confirmed by others.

Abnormal spatial discrimination has been described in focal hand dystonia and cervical dystonia with disorganized cortical somatotopy in focal hand dystonia. Treatment with botulinum toxin temporarily improves abnormal spatial discrimination in cervical dystonia and reverses the abnormal motor cortical representation of the hand in cervical dystonia; whereas intensive rehabilitation normalizes cortical sensory somatopy in focal hand dystonia. The improvement of these abnormalities in response to symptomatic therapies suggests that they represent secondary adaptation to disease manifestation.
A candidate mediational endophenotype, abnormal temporal discrimination threshold, is not altered by symptomatic therapy for dystonia, including deep brain stimulation (DBS)\textsuperscript{27} and botulinum toxin therapy.\textsuperscript{28}

Despite recent emphasis on abnormalities of cortical function detected by various techniques as primary abnormalities in AOPTD,\textsuperscript{29,30} we suggest that these cortical abnormalities in AOPTD probably represent epiphenomena. Secondary dystonia is not seen in patients with discrete cerebral cortical lesions and the argument that primary dystonia reflects disordered basal ganglia circuitry has been clearly stated.\textsuperscript{31}

**Trait Versus STATE in DYT1 Dystonia**

The differences between trait and state measures are most easily assessed in DYT1 and DYT6 dystonia where non-manifesting carriers (NMC) can be clearly defined. Using \textsuperscript{18}F fluorodeoxyglucose positron emission tomography (FDG-PET), Carbon et al.\textsuperscript{32} and Carbon and Eidelberg\textsuperscript{33} compared patterns of regional metabolic activity between NMC and clinically affected DYT1 and DYT6 carriers. Manifesting DYT1 and DYT6 patients showed a pattern of increased activity called the dystonia manifestation-related pattern (DYT-RP) whereas NMC of either genotype showed reduced DYT-RP expression. Manifesting carriers had developed abnormal cortical sensorimotor integration. Thus, by using FDG-PET, penetrance-related increased metabolic activity in the cortex (a secondary endophenotype) was distinctly different from the genotype-related effects.

In an earlier study comparing NMC and manifesting carriers of the DYT1 gene, both groups of carriers were found to have significant reduction in intracortical inhibition but the NMC participants had normal spinal reciprocal inhibition.\textsuperscript{34} The authors noted that the cortical abnormalities in the NMC were in pathways influenced by basal ganglia output and that the additional abnormalities in the manifesting DYT1 patients may be secondary to disease manifestation affecting connected structures.

The evidence that some electrophysiological abnormalities are secondary to the movement disorder is supported by findings that dystonia patients, with various phenotypes, treated successfully with pallidal DBS, postoperatively show slowly improving markers of impaired cortical inhibition that correlate to clinical response and early response to plasticity protocols.\textsuperscript{35} In contrast, abnormal temporal discrimination is not corrected by DBS for dystonia despite clinical improvement.\textsuperscript{27}

**TDT as a Mediational Endophenotype in AOPTD**

A number of putative endophenotypes have been identified for AOPTD.\textsuperscript{11} We and others have examined the utility of the TDT as a valid mediational endophenotype in both familial and sporadic AOPTD.\textsuperscript{36-44} The TDT is defined as the shortest time interval in which 2 stimuli may be determined as asynchronous (normally about 30–40 ms). The TDT is regarded as abnormal if $>2.5$ SD above the control mean (TDT $Z$-score $>2.5$). Abnormal TDTs show autosomal dominant transmission in multiplex AOPTD families\textsuperscript{43} and in families of sporadic AOPTD patients.\textsuperscript{42} Unaffected obligate heterozygotes have abnormal TDTs.\textsuperscript{43} The TDT is abnormal in more than 80% to 90% of patients with various AOPTD phenotypes, and sensitivity is highest (97%) in the most common phenotype, cervical dystonia.\textsuperscript{44} The specificity of an abnormal TDT in various studies is 98% to 100%. There is age-related penetrance of an abnormal TDT in unaffected first-degree relatives with full penetrance by 48 years of age in women and 40%
penetrance in men. Unaffected relatives with abnormal TDTs have larger putaminal volumes by voxel-based morphometry (VBM) than relatives with normal TDTs. An abnormal TDT is not specific to AOPTD, having been found also in other basal ganglia disorders and NMC of DYT1 dystonia and PINK1 Parkinsonism.

The finding of abnormal TDTs in these other basal ganglia disorders may be regarded by some as a weakness in the hypothesis that abnormal temporal discrimination is a mediational endophenotype in AOPTD. However, the presence of abnormal TDTs in diseases with obviously abnormal dopamine processing supports a thesis that temporal discrimination is a marker of a disturbed basal ganglia circuit involving dopamine processing in the putamen (described in Fig. 4).

Apart from scientific curiosity, the concept an abnormal TDT as a mediational endophenotype has a number of important implications for our understanding of the pathogenesis of AOPTD.

**The Mediational Endophenotype Concept as a Hypothesis Generator**

Five hypotheses arise from considering abnormal temporal discrimination as a mediational endophenotype in AOPTD (Table 1).

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**FIG. 2.** The concept of the mediational endophenotype (abnormal temporal discrimination) applied to adult onset primary torsion dystonia (AOPTD) and environmental factors that affect penetrance of the phenotype. Age-related full penetrance of the endophenotype develops in unaffected first-degree female relatives by 48 years of age with 40% penetrance in male relatives. The phenotype is poorly penetrant and depends on age and other environmental factors including trauma, overuse (in focal hand dystonia and musicians), and eye disease (in blepharospasm). There is evidence that coffee drinking is protective. Trauma may not be an environmental risk factor for AOPTD penetrance; an increased propensity to trauma may be a result of having abnormal temporal discrimination. Whether all AOPTD is genetic is not clear: the yellow arrow suggests that there may be a mechanism whereby some environmental agents (overuse) may cause focal dystonia without preceding genetic liability.

**FIG. 3.** The postulated input to the subcortical basal ganglia circuit involved in the detection of salient unexpected environmental change and in the temporal discrimination task (from Peter Redgrave with permission). Direct retinal input to the superior colliculus affects branched projections to the intralaminar thalamic nuclei and to the substantia nigra pars compacta. Converging phasic inputs to the striatum from the thalamic intralaminar nuclei (GLU, glutamate) and substantia nigra (DA, dopamine) signal unexpected salient environmental change and affect striatal output (see Fig. 4).
**Trait Versus State**

An endophenotypic marker in non-manifesting gene mutation carriers is purely genotype-specific whereas a phenotypic marker may be a secondary disorder of cerebral function. The advantage of examining trait rather than state-dependent abnormalities is that the trait abnormality is linked to genetic inheritance and more probably relates to the underlying disease pathogenesis.

**The Endophenotype as a Tool to Explore Environmental Factors in AOPTD**

If the endophenotype, an abnormal TDT, is fully penetrant and the phenotype, AOPTD, only weakly penetrant, one might compare antecedent environmental exposures between clinically affected individuals and their similarly aged, unaffected, siblings (aged 40 years or older) who have abnormal TDTs in a matched phenotype-endophenotype study. Previous studies of environmental factors in disease expression in AOPTD have compared affected individuals with unaffected first-degree relatives or with non-family members who have other, nondystonic, disorders. The power and specificity of a study of environmental factors would be considerably increased by comparing affected AOPTD patients with their similarly aged, or older siblings who have abnormal TDTs (postulated to be non-manifesting gene carriers) (Fig. 2). Comparing the history of various environmental exposures, for example to peripheral trauma, in 3 groups: (1) patients with AOPTD; (2) their siblings of similar ages with normal TDTs; and (3) siblings with abnormal TDTs, may determine those environmental exposures that influence the risk of developing AOPTD.

**The Endophenotype in Gene Discovery**

It is likely that AOPTD is genetically heterogeneous with a number of different genetic mutations causing similar phenotypes. Informative multiplex AOPTD families are rare. Studies on multiplex families are likely to be productive using exome sequencing techniques (as has recently been demonstrated). The use of a valid, reliable endophenotype in multiplex families, by indicating gene carriage in unaffected family members, might facilitate successful linkage analysis or exome sequencing. Cosegregating single-nucleotide polymorphism variants identified in multiplex AOPTD families may be screened in other family members with and without abnormal TDT scores to validate the endophenotype. A validated TDT endophenotype could then be used in the further examination by linkage analysis of multiplex families with few affected individuals.

**The Mediation Endophenotype, Abnormal TDT, Indicates the Pathogenetic Mechanisms of AOPTD**

Understanding the anatomy and physiology of normal temporal discrimination and the pathophysiology of an abnormal TDT may indicate novel pathogenic mechanisms of AOPTD. Evidence supporting the role of an abnormal TDT in the pathogenesis of AOPTD includes structural and functional abnormalities implicating the putamen and, more specifically, disordered dopamine processing. This evidence includes:

1. Putaminal enlargement in patients with AOPTD and musicians with task-related dystonia. Unaffected first-degree relatives of AOPTD patients with abnormal TDTs also have putaminal enlargement compared to relatives with normal TDTs. It is to be noted that putaminal hypertrophy seems to be associated with dysfunction (unlike in the cortex where atrophy is associated with dysfunction). For example, in musicians there is a relationship between worsening performance of key-stroke and increased putaminal size. However, the relationship between AOPTD and putaminal hypertrophy has recently been questioned.

2. Perceptual certainty in a temporal discrimination task is associated with putaminal activation; the putamen is involved early in a temporal processing task.

3. Reduced putaminal dopamine 2 receptor (D2R) availability is found in cranial dystonia, focal hand dystonia, and in manifesting and NMC of both DYT1 and DYT6 dystonia. One required experiment would be to assess D2R availability in AOPTD patients and their unaffected siblings with abnormal TDTs in comparison to their similar-age siblings with normal TDTs.

4. Abnormal temporal discrimination is found in Parkinson’s disease and is improved by l-dopa replacement but not by subthalamic nucleus DBS.

**Abnormal Temporal Discrimination as a Mediation Endophenotype Indicates a Disordered Subcortical-Basal Ganglia Circuit in AOPTD**

The hypothesis is that normal temporal discrimination is a marker of the integrity of an alerting system to novel environmental change. Normal temporal discrimination can detect changes in the visual and tactile...
environment within a remarkably defined short interval of approximately 30 to 40 ms and may have evolutionary significance. Such an alerting mechanism would be of use in detecting prey or avoiding a predator (Fig. 3). Dopamine plays a pivotal role in motivational control and reward processing but its role in alerting organisms to novel environmental events has only recently been recognized. 69 Mid-brain dopaminergic neurons exhibit short-latency responses to biologically salient events.70

The Superior Colliculus: Detecting and Selecting Salient Stimuli for Reaction

The superior colliculus is a laminated structure with a superficial purely sensory layer responding to visual inputs directly from the retina. Neurons in the deeper layers respond to multisensory, visual, auditory, and tactile inputs.71,72 There is increasing evidence that superior colliculus activity is central to the detection of unpredictable, biologically salient events that can trigger interruption of ongoing behavior, contribute to higher-order decision making, and initiate orienting responses of the head and eyes.73

The anatomical pathway by which salient environmental changes influence motor responses involves a subcortical basal ganglia loop.72,74,75 The superior colliculus is the primary source of short-latency visual inputs to the dopaminergic cells of the substantia nigra pars compacta (SNpc)76 and the intralaminar thalamic neurons (which innervate the cholinergic striatal interneurons77); this pathway has been demonstrated in the rat78 and in primates.79,80

It has been known for some time that the main output from the intralaminar thalamic nucleus is to the cholinergic interneurons of the striatum.81 These neurons in behaving primates respond to unexpected visual, auditory, or somatosensory stimuli with brief
short-latency firing, signaling biologically salient events unrelated to reward. Although neurologists have often regarded the interplay of dopamine and acetylcholine in the striatum as apparently antagonistic (in Parkinson’s disease for example), more recent sophisticated in vitro studies have shown that synchronous stimulation of cholinergic interneurons dramatically elevates striatal dopamine release. Also, activation of glutaminergic thalamic inputs to the striatum evokes dopamine release.

Recent experimental work in animals has demonstrated that thalamic stimulation excites a burst-and-pause response in the cholinergic interneurons of the striatum. The resultant burst of acetylcholine release causes a brief decrease in corticostriatal transmission to medium spiny neurons in both the direct and indirect pathways, via activation of postsynaptic M1 receptors on medium spiny neurons in the indirect pathway. This provides a mechanism for cessation of ongoing activity in response to a biologically salient event and, if required, a redirection of behavior appropriate to the environmental change that has been detected.

**Temporal Discrimination: A Measure of the Detection of Salient Environmental Change**

In humans, the temporal discrimination threshold is a measure of the efficacy of detection of salient environmental change and is a function of the collicular-thalamic-putaminal-pallidal-nigral loop described in Figure 4. Undoubtedly temporal discrimination is also later processed by cortical areas, but the weight of evidence adduced above indicates that the TDT task in our experimental paradigm is largely a function of this subcortical basal ganglia pathway.

Predators and prey are unlikely concerns for the urbanized human. However, accurate detection of looming objects and estimation of time to collision are important functions for safety in our daily lives, whether as cyclists, pedestrians, or drivers (Fig. 4). In an fMRI study in humans, whereas timing computations for collision events were functions of the anterior insula, the detection of the looming stimulus (opposed to a receding stimulus) was associated with activation of both superior colliculi. It is possible that the association of peripheral trauma with later development of AOPTD may not be causal and that individuals with prolonged temporal discrimination also have reduced ability to detect in a short time a salient environmental stimulus and thus are less able to avoid trauma.

**Conclusion**

Mediational endophenotypes may be powerful tools to advance our understanding of the pathogenesis of AOPTD. Clearly, the ultimate validation of abnormal temporal discrimination as a mediational endophenotype of AOPTD will depend on gene discovery, but the accumulating indirect evidence is compelling. Future research on the pathogenesis of AOPTD should be focused on the disrupted subcortical–basal ganglia pathway implicated by abnormal temporal discrimination.

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**References**


