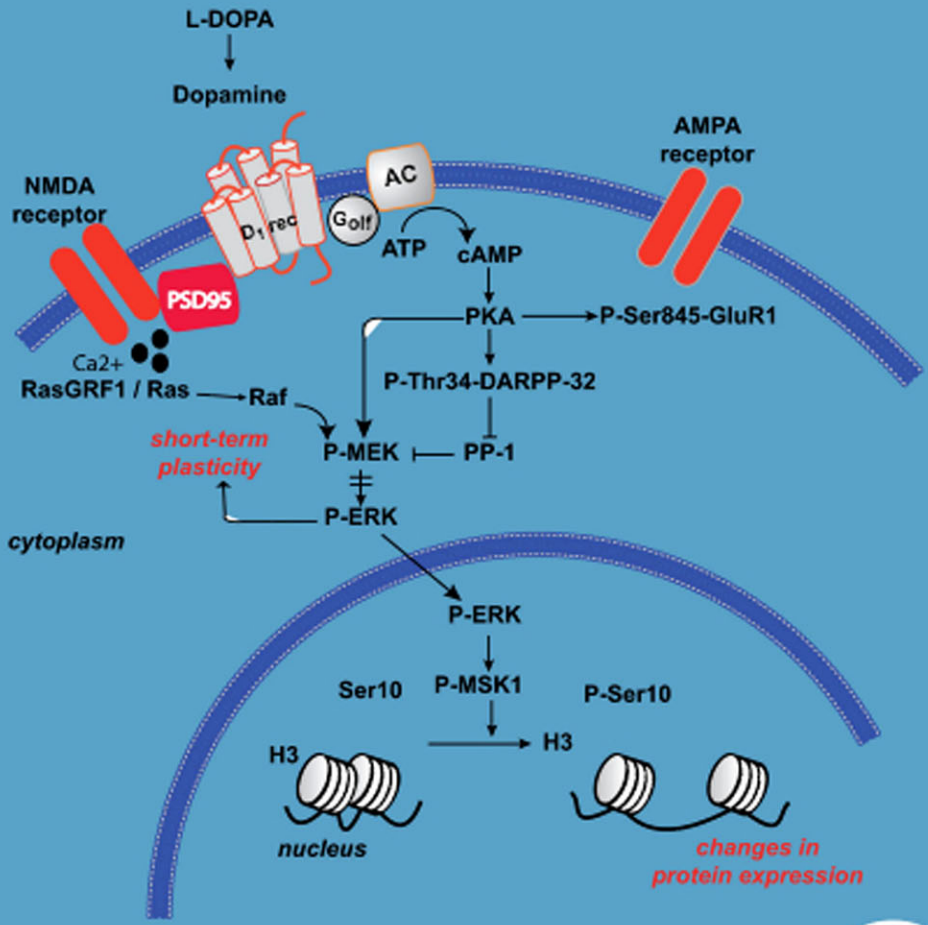
# International Review of Neurobiology

## Pathophysiology, Pharmacology, and Biochemistry of Dyskinesia

Volume 98




Edited by
Jonathan Brotchie, Erwan Bezard
and Peter Jenner

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# Pathophysiology, Pharmacology, and Biochemistry of Dyskinesia

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Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London, NW1 7BY, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
Linacre House, Jordan Hill, Oxford OX2 8DP, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

First edition 2011



ISBN: 978-0-12-381328-2
ISSN: 0074-7742



Printed and bound in USA

11 12 13 14 10 9 8 7 6 5 4 3 2 1

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# PREFACE

Dyskinesia is a clinical phenomenon that sounds simplistic in describing a disruption of motor function leading to the expression of involuntary movements but the terminology hides a multitude of signs and symptoms that underlie both drug induced and genetic/idiopathic diseases where dyskinesia is expressed. At an initial level dyskinesia is composed of chorea, dystonia, and athetosis that can be focal, segmental, or generalized in nature. Then add to this the many manifestations of tics, ballisms, and the motor and mental components of akathisia together with the underlying causes of psychogenic movement disorders, and you find that dyskinesia is anything but simplistic in nature.

Since some causes of dyskinesia are well known, such as neuroleptic involvement in acute dystonia and tardive dyskinesia and the chorea and dystonia characterizing L-dopa-induced dyskinesia in Parkinson's disease, it might be thought that the underlying mechanisms responsible for dyskinesia would be thoroughly understood. Similarly, in a disease such as Huntington's chorea where pathological change is well documented, clues to those regions of brain and those specific nuclei involved in the initiation and expression of dyskinesia might be very obvious. But, while advances in understanding have been made, there are many unknowns when considering the pathophysiology of the array of dyskinesias that afflict man. Even in well-defined familial diseases, such as the inherited dystonias and Huntington's chorea, the identification of the gene products responsible for onset involuntary movements has not taken us directly to their cause. When considering what appear to be entirely sporadic dyskinesias, including many of the dystonias and tics, the situation becomes even less well understood.

It is this lack of knowledge that has held back the introduction of effective pharmacological treatments for dyskinesia. However, the role of genetic predisposition, specific neuronal cell groups and specific neurotransmitter systems is now better understood than previously and this is opening up new avenues for drug treatment and also for the avoidance of the induction of dyskinesia where these are drug related in nature. Fortunately, surgical approaches to the treatment of dyskinesias and the use of botulinum toxin have advanced significantly in some areas and these start to offer the opportunity for the provision of relief from constant involuntary movement. This volume is a comprehensive review of the current

state-of-the-art in relation to the major manifestations of dyskinesia occurring in many different clinical settings. The contributors are the leaders in their fields and they have reviewed the causes, clinical symptomatology, pathology and biochemistry and treatment, current and future, for this important group of movement disorders. The volume offers a big picture overview of dyskinesia and it will be essential reading for neurologists, geriatricians, psychiatrists, and neuroscientists who face the clinical spectrum of dyskinesia on a daily basis.

# AN INTRODUCTION TO DYSKINESIA—THE CLINICAL SPECTRUM

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The term movement disorder is used to describe a variety of abnormal movements, and may involve an excess or paucity of movement. Careful characterization of phenomenology is an essential component of diagnosis. Factors such as speed, amplitude, duration, distribution, rhythmicity, suppressibility and pattern of movement provide valuable information to guide the clinician in their assessment of the movement disorder. In this chapter, the clinical spectrum and phenomenology of dyskinesias will be reviewed.

## I. Introduction

Movement disorders may present as either an abnormal excess or paucity of movement. Commonly used terms for excessive movements include dyskinesia, hyperkinesia, and abnormal involuntary movement. These terms are often used interchangeably. Terms used to describe paucity of movement include hypokinesia, bradykinesia, and akinesia.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00001-8





0074-7742/10 $35.00

Movements may also be categorized according to the degree of volitional control. As such, they may be divided into automatic, voluntary, semi-voluntary (or "unvoluntary") (Fahn and Jankovic, 2007a; Fahn, 2005; Tourette Study Group, 1993) and involuntary (Jankovic, 1992). Automatic movements are learned motor behaviors that are performed without conscious effort such as walking (Fahn and Jankovic, 2007a). Voluntary movements are those that are performed under volitional control, and are intentionally planned. Semi-voluntary (or "unvoluntary") movements are induced by an inner sensory stimulus or compulsion, and are performed in order to relieve the unpleasant or unwanted sensation. Semi-voluntary movements, such as some tics, are usually suppressible. Involuntary movements, such as myoclonus, are usually not suppressible, but most other hyperkinesias are at least partially suppressible and often disappear completely during sleep.

Evaluation of abnormal movement first involves determination of whether they are involuntary, voluntary, semi-voluntary, or automatic. For instance, exaggerated gestures, compulsive movements, and mannerisms should be differentiated from involuntary movements. The movements are then characterized phenomenologically. Features such as speed, amplitude, duration, distribution, rhythmicity, and pattern of the movements are observed including the presence of simple versus complex movements. Movements can be intermittent (paroxysmal) or continuous (repetitive without stopping). Induction, which refers to whether the movements are stimulus-induced, action-induced, or exercise-induced may also assist with the characterization of the movements. The examiner should also assess for suppressibilty by volitional attention or sensory tricks (Fahn and Jankovic, 2007a) and whether there are premonitory localized sensations or more generalized urges prior to execution of the movement such as that which occurs in tics (Kwak *et al.*, 2003). Once the movement is characterized by phenomenology, the next step is to determine the etiology of the particular movement disorder.

This chapter will focus on phenomenology and the clinical spectrum of dyskinesias, the critical elements in the diagnosis of movement disorders. To enhance the understanding and recognition of the broad phenomenology of hyperkinetic movement disorders the reader is invited to review textbooks on movement disorders that are accompanied by videos (Albanese and Jankovic, 2011; Fahn *et al.*, 2011; Jankovic and Tolosa, 2007). The sequence of the various dyskinesias is organized alphabetically. A summary of the key clinical features of each movement disorder is provided in the table.

## II. Akathisia

Akathisia is used to describe an abnormal subjective state of restlessness and an urge to move. It is accompanied by restless movements, which give temporary relief to the inner sense of restlessness. Most movement disorder experts consider akathisia

as a combination of sensory components (subjective feeling of inability to be still) and motor components (restless, complex, stereotypic movements). Akathisia may be acute and self-limiting, but has also been recognized as a persistent and late-onset phenomenon, particularly in the setting of a tardive syndrome (Braude and Barnes, 1983; Burke *et al.*, 1989; Fahn, 1978; Weiner and Luby, 1983).

The subjective aspect of akathisia consists of an inner sense of restlessness or tension, associated with an aversion to remaining still (Fahn and Jankovic, 2007e). It may also present as focal areas of discomfort, particularly involving the oral or genital region. The motor component of akathisia consists of "akathitic" movements that are generally complex, stereotypic, semi-purposeful, and repetitive (Fahn and Jankovic, 2007e). The legs appear to be most frequently affected, although other body parts may also be involved. Observations of patients with tardive akathisia reveal a number of characteristic movements, including body rocking, alternating ("pumping") movements of legs or crossing–uncrossing of legs when sitting, shifting weights from one foot to another when standing, walking on the spot, and other complex movements (Braude and Barnes, 1983). A study of patients with tardive akathisia also found involvement of complex hand movements such as touching the scalp, face rubbing, or scratching, as well as truncal rocking and respiratory grunting and moaning (Burke *et al.*, 1989). Akathisia needs to be differentiated from other conditions, such as restless legs syndrome, in which similar symptoms are described, but are mainly localized to the legs and are predominantly nocturnal (Blom and Ekbom, 1961).

## III. Ballism

Ballism refers to forceful, flinging, high-amplitude movements that mainly involve the proximal limb muscles. When unilateral, it is referred to as hemiballism. Choreiform movements often co-exist with ballism, and as such they may be considered as variants in the same clinical spectrum (Dewey and Jankovic, 1989). Hemichorea-hemiballism can be observed with contra-lateral basal ganglia pathology, particularly involving the subthalamic nucleus, but damage to other parts of the brain have also been associated with this syndrome.

## IV. Chorea

Chorea, derived from the latin word "to dance," results from dysfunction in the complex neuronal networks interconnecting motor cortical areas with the basal ganglia (Cardoso *et al.*, 2006). Thomas Sydenham reported childhood chorea in the late 17th century, however the association between rheumatic fever and Sydenham's chorea had not yet been established at that stage (Cardoso *et al.*,

2006). In the 19th century, George Huntington's concise and detailed descriptions of hereditary chorea on affected families in the state of New York, later known as Huntington disease, further helped to define this entity (Huntington, 1872).

Chorea refers to involuntary, irregular, purposeless, non-rhythmic, abrupt, and unsustained movements that seem to flow randomly from one body part to another. It is differentiated from other rapid and non-rhythmic hyperkinetic movement disorders by its random, un-patterned, and non-repetitive nature. Infrequent and mild chorea may appear as isolated, small-amplitude brief movements. It may resemble restless, fidgety, or anxious behavior. Although slower than myoclonus, mild chorea can also be difficult to distinguish from myoclonic movements (Fahn and Jankovic, 2007c). When chorea is more severe, it may appear almost continuous, flowing from one site of the body to another. The term parakinesia refers to the incorporation of the involuntary movements into semi-purposeful movements, in an attempt to camouflage them. Chorea may be partially suppressible. Chorea is often associated with motor impersistence, in which the individual is unable to maintain a sustained contraction. Examples include an inability to maintain prolonged tongue protrusion and handgrip ("milkmaid grip"). Rating scales, such as the UHDRS, allow some degree of quantification of the distribution and severity of the chorea and motor impersistence.

The prototype for chorea is seen in Huntington disease. Early surface EMG studies in patients with Huntington disease revealed a wide variability in the timing of EMG burst duration, with bursts even as brief as 10–30 ms (Hallet and Kaufman, 1981). In addition, there was a continuous change in the activation order of each muscle, as well as changes in the type of movement within an individual muscle (Marsden *et al.*, 1983). Some studies found a tendency for synchronous activation between antagonist muscles (Hallet and Kaufman, 1981; Rondot, 1977). Surface EMG in patients with Sydenham chorea revealed asynchronous bursts of activity in antagonist muscles without reciprocal inhibition (Hoefer and Putnam, 1940). Variation was also seen in the timing of EMG burst duration (Herz, 1944).

Chorea is typically bilateral. Focal chorea or hemichorea may raise the suspicion of an underlying structural lesion, although hemichorea or asymmetric chorea is common in patients with Sydenham disease, a term preferred to Sydenham chorea as other neurological and behavioral symptoms, besides chorea, are often present (Wang *et al.*, 2001).

## V. Dystonia

Dystonia is described as a syndrome of involuntary sustained muscle contraction, causing twisting or repetitive movements, or abnormal postures (Fahn, 1988;

Fahn *et al.*, 1998). The twisting quality is unique to this condition, and helps to distinguish dystonic postures from other conditions that are associated with increased muscle tone. An exception to this is when the nature of the joints is such that they do not allow twisting, such as in the jaw and facial muscles (Fahn and Jankovic, 2007b). The movements in dystonia are patterned, repeatedly involving the same muscle groups. Dystonia involves simultaneous contraction of agonist and antagonist muscles, and electrophysiological studies reveal predominantly prolonged co-contracting activity (Herz, 1944; Yanagisawa and Goto, 1971).

Dystonia can be classified according to its distribution (Fahn and Jankovic, 2007b). Focal dystonia refers to involvement of a single area of the body such as blepharospasm or torticolis. When a continguous body part is also affected, the term segmental dystonia is used. Generalized dystonia refers to segmental crural dystonia, such as involvement of both legs or leg plus trunk, in addition to any other part of the body. Multifocal dystonia is used when two or more non-continguous parts of the body are affected. When there is unilateral involvement, the term hemidystonia is used. There is a great variation in the speed at which the involuntary movements occur in dystonia. Dystonic spasms refer to brief dystonic contractions. The term dystonic posturing describes more prolonged movements lasting minutes to hours. When present for weeks or longer, they may lead to fixed contractures (Fahn and Jankovic, 2007b).

Dystonia may also be classified as primary or secondary (Müller, 2009). Primary dystonia classically presents as a pure or predominant dystonia syndrome, and is frequently caused by a single gene. DYT1 dystonia, otherwise referred to as idiopathic torsion dystonia, is an example of primary dystonia. Classically, it presents as a childhood onset dystonia with progressive generalization, although the clinical phenotype may vary greatly. Dystonia plus syndromes, which are also usually monogenic in origin, refer to conditions in which additional neurological manifestations are present. An example includes myoclonus dystonia, or DYT11, an alcohol responsive condition in which the onset of myoclonic jerks typically precede the appearance of relatively mild dystonia. Hereditary progressive dystonia with marked diurnal fluctuation, or Segawa disease, is another example of a dystonia-plus syndrome (Segawa, 2011), and is associated with parkinsonism. It is characterized by marked diurnal fluctuation during childhood. Clinically, it may present as either a postural or action type, depending on the locus of the mutation, or other factors. The postural type is more common, classically starting in the lower limb during childhood. The condition tends to improve during the third decade, and stabilize in the forth decade. On the other hand, the action dystonia type of childhood onset has additional features of action dystonia affecting the upper limb or neck. Dopa-responsive dystonia is caused by mutation of the GCH-1 gene. Secondary dystonias are those that occur in the setting of another disorder.

Primary dystonia typically starts as a focal dystonia. The anatomic presentation of primary torsion dystonia is influenced by the age at onset, with a distal to proximal progression in the distribution of the dystonia with increasing age (O'Riordan *et al.*, 2004). In the majority of cases, the dystonia remains localized. Dystonia that occurs at a younger age of onset is generally associated with a higher risk of progression (Ozelius and Bressman, 2011). When onset is in childhood or adolescence, it tends to start in the arm or leg, and then progress to other body parts over the course of 5–10 years. Spread of dystonia usually occurs by affecting continguous body parts, resulting in segmental dystonia. Progression to generalized dystonia is more likely in childhood-onset dystonia (Fahn, 1986; Marsden *et al.*, 1976). Dystonia which starts in adulthood is typically focal or segmental, and may often present as blepharospasm, oromandiular dystonia, and torticollis (Le *et al.*, 2003). Lower limb dystonia that occurs in adulthood is usually secondary to other causes, although adult onset primary lower limb dystonia has been described in small series (Schneider *et al.*, 2006).

Action dystonia refers to the appearance or exacerbation of dystonia during voluntary movements. Primary dystonia typically starts as a specific action dystonia, and may not be present at rest. This is in contrast to secondary dystonias, which are more likely to begin with dystonia at rest (Svetel *et al.*, 2004). Task-specific dystonias, a type of action dytonia, occur almost exclusively with a particular task. Task-specific dystonia more commonly affects the upper limbs and face. They tend to occur in highly skilled, over-learned tasks (García-Ruiz *et al.*, 2010). The most common adult-onset upper limb task-specific dystonia is writer's cramp (Pont-Sunyer *et al.*, 2010). Musician's cramp occurs whilst playing a musical instrument (Jankovic and Ashoori, 2008). Embouchure dystonia affects the control of the lip, jaw, and tongue muscles, and may be seen in woodwind and brass players (Frucht *et al.*, 2001). The lower limbs are rarely affected, although they have been reported in professional dancers (García-Ruiz *et al.*, 2010). Isolated reports of task specific dystonia have also been described with walking down steps, suggesting that relatively autonomic functions may also be affected (Lo and Frucht, 2007). Runner's dystonia has been reported to occur initially in long-distance running, and later progressing to also involve walking (Wu and Jankovic, 2006). One case of runner's dystonia involving the neck and trunk reportedly responded to an interoceptive sensory trick with mental imagery (Suzuki *et al.*, 2011).

As the condition progresses, dystonia may occur with less specific voluntary action. When the dystonia is also induced by action in other parts of the body, it is referred to as overflow dystonia. In the more severe stages, the dystonia may also be present at rest. The so-called paradoxical dystonia is an uncommon phenomenon in which rest dystonia improves with voluntary movements (Fahn, 1989). The most common example of paradoxical dystonia is blepharospasm. Approximately 60% of patients experience improvement with talking, whilst 40% experience worsening of blepharospasm (Fahn, 1985).

Dystonia may be exacerbated by fatigue and stress. Dystonia gravidarum refers to the uncommon occurrence of dystonia during pregnancy (Fasano *et al.*, 2007; Lim *et al.*, 2006). Relieving factors for dystonia may include sleep and relaxation. The sensory trick, or geste antagoniste, is a unique feature of dystonia, in which the dystonic movement can be diminished with a tactile or proprioceptive stimulus. Examples of the sensory trick include resting the hand on the chin or side of face to improve cervical dystonia. One study found that the application of a sensory trick leads to changes in cortical activation patterns, and possibly modulates motor programming (Naumann *et al.*, 2000).

Rhythmic group action potentials may occur in dystonia, resulting in the presence of a dystonic tremor (Jedynak *et al.*, 1991). The tremor may only appear when the affected body part is placed in a position which is opposite to the abnormal dystonic contractions, and disappear when the body part is moved to the position favored by the dystonia (Fahn and Jankovic, 2007b). Dystonic tremor has a more irregular quality compared to essential tremor. Apart from tremor, the presence of other associated movement disorders, such as myoclonus or parkinsonism, raises the suspicion of secondary dystonia. (Jedynak *et al.*, 1991). Fixed painful dystonia, which is usually post-traumatic, is possibly related to chronic regional pain syndrome (Schrag *et al.*, 2004). Evidence of higher motor dysfunction has been reported in one study with primary cervical dystonia, whereby significantly more errors were made in copying meaningless hand gestures, compared with a control group (Hoffland *et al.*, 2010).

## VI. Jumpy Stumps

The term jumpy stumps is used to refer to the involuntary movement disorder that may be seen in the stump of an amputated limb. The movements are varied, and may consist of jerking, tremulousness, or spasms (Steiner *et al.*, 1974). They may be associated with severe phantom pain. A case of psychogenic jumpy stumps has also been reported (Zadikoff *et al.*, 2006).

## VII. Levodopa-Induced Dyskinesias

The common clinical presentations of levodopa-induced dyskinesias include peak-dose (on) dyskinesia, diphasic dyskinesia, and off-dystonia (Voon *et al.*, 2009). Levodopa-induced dyskinesias are more likely to start on the side more severely

affected by Parkinson's disease (PD). A retrospective study found that dyskinesias may also start bilaterally, or in the cranio-cervical region, in a small proportion of patients (Fabbrini *et al.*, 2009).

Peak dose dyskinesia typically coincides with the on-response following levodopa administration. This is the most common temporal pattern of levodopa-induced dyskinesia (Encarnacion and Hauser, 2008). It is aggravated by dopaminergic medication, and usually improves as the treatment wears off. Movements are predominantly stereotypic or choreiform in nature, particularly in the earlier stages of levodopa therapy (Fahn, 2000). Mild levodopa-induced chorea tends to be non-disabling, and is often first noticed by family members rather than by the patients themselves. The mildest form is that of "action" chorea (Fahn, 2000), in which the involuntary movements, such as continuous head bobbing, occur only during active voluntary movements, such as talking or walking. The chorea in peak dose dyskinesia typically involves the neck, axial, and upper limb muscles. Ocular dyskinesias (LeWitt, 1998; Linazasoro *et al.*, 2002) and belly dancer's dyskinesias have also been described (Carecchio *et al.*, 2010). Ballism occurs uncommonly in levodopa-induced dyskinesia (Hametner *et al.*, 2010). When present, it usually occurs in the setting of severe chorea, rather than as an isolated abrupt flinging of the limbs. In the later stages of dopaminergic therapy, patients may develop more dystonia and less chorea, and this tends to be more disabling. The combination of dystonia and chorea may manifest as some sustained posturing at the height of a movement. Peak-dose dyskinesia can also exist as a pure dystonia, without accompanying chorea. The sustained contraction is typically limited to one part of the body such as an arm, leg, or trapezius muscle. It may also affect the oral, lingual, and palatal muscles, resulting in disturbances in articulation. Peak-dose dytonia occurring with relatively low doses of levodopa raises the suspicion of multiple systems atrophy. Some patients develop dyskinesia as soon as they attain a medication response ("on-response"), and this lasts until the medication wears off. In this so-called "square wave response," the dyskinesia always accompanies the on-phase, and patients are unable to be "on" without associated dyskinesia. In one study, the temporal relationship between the antiparkinsonian response to an intravenous infusion of levodopa was essentially identical to the onset and offset of dyskinesia (Nutt *et al.*, 2010). This was observed in both PD patients with dyskinesias on long-term levodopa therapy, as well as those who were previously untreated with levodopa in whom dyskinesias first appeared during the study follow-up period. The time to onset of dyskinesia and antiparkinsonian response tended to be shorter in those on longer-term levodopa therapy.

Diphasic dyskinesia appears at the beginning of the medication effect, prior to the attainment of a full medication response. It may also reappear as the medication effect starts to wear off. It develops as the plasma levels of levodopa are rising or falling, but not during peak plasma level (Fahn, 2000; Lhermitte *et al.*, 1978; Muenter *et al.*, 1977). Originally, these were referred to as "D-I-D", a term coined

by Muenter and colleagues in reference to the temporal pattern of dystonia (dyskinesia)-improvement-dystonia (dyskinesia)(Fahn, 2000; Muenter *et al.*, 1977). Diphasic dyskinesias typically consist of repetitive stereotypic, large-amplitude, rhythmical involuntary movements. They may have ballistic movements (Hametner *et al.*, 2010). The dyskinesias can be explosive and distressing (Marconi *et al.*, 1994). Surface electromyographical studies reveal alternating activation of antagonist muscles (Fahn, 2000; Voon *et al.*, 2009). Many patients may also experience dystonia, chorea, or a mixture of the two (Fahn, 2000). Diphasic dyskinesias predominantly affect the legs. They typically start in the foot that is most affected by disease. The dyskinesias may then spread as an "ascending wave" to the contralateral side, the trunk, upper limbs, and head (Marconi *et al.*, 1994). In one study using video-electromyographic recordings, an evolution of different types of dyskinesias was seen, including a transition from dystonic to choreic movements (Marconi *et al.*, 1994). In addition, the changes in the characteristics of the dyskinesias were simultaneous with the change in spatial distributions. Diphasic dyskinesias may co-exist with parkinsonian features such as tremor or hypomimia, particularly in the upper limbs or face.

Off-period dystonias occur when the dopaminergic benefit has worn off, as in early morning, leaving the patient in an "off" state without medication effect. Early morning dystonia affecting the feet is often quite painful and is one of the most common presentations of "off-period" dystonia, occurring as the previous night's dose of levodopa has completely worn off (Melamed, 1979). This sign has been incorporated into the Unified Parkinson's Disease Rating Scale as an assessment of motor fluctuation. "Off-period" dystonia may, however, also occur during any "off period", and may be segmental or generalized. The dystonia itself consists of a prolonged sustained contraction, and is often distressful to the patient. In one study, off-period dystonia was found to be continuous with diphasic ("onset-of-dose") dykinesias in 8 out of 11 patients (Marconi *et al.*, 1994). A number of differences were noted between off-period and diphasic ("onset-of-dose") dyskinesias. The contractions of muscle groups in off-period dystonia were more sustained and relatively fixed compared with the more vivid movements in diphasic dyskinesia. Furthermore, the side of maximal prevalence was not always concordant between the two types of dyskinesias, and the passage from off-period dystonia to diphasic dyskinesias was not constant (Marconi *et al.*, 1994).

Myoclonus (see below) has also been described in the setting of levodopa. It typically signifies levodopa toxicity, but its presence should also raise the suspicion of some other form of Parkinsonism such as diffuse Lewy-body disease (Fahn, 2000). Levodopa associated myoclonus has also been described in the setting of sub-maximal dopamine levels, occurring during the first 10–20 min after levodopa administration, and disappearing as the parkinsonian symptoms improve (Klawans *et al.*, 1975; Marconi *et al.*, 1994). Levodopa associated myoclonus typically causes brief muscle contractions (positive myoclonus) rather than

inhibitions of muscle activity (negative myoclonus) as is seen in asterixis. It usually manifests as a single jerk in the extremities, either unilaterally or bilaterally. It is predominantly nocturnal, but may also occur during the day.

Akathisia has been reported to occur in some patients in the period preceding the onset of levodopa benefit (Marconi *et al.*, 1994).

Respiratory dyskinesias are an uncommon form of levodopa-induced dyskinesia, and are characterized by episodes of symptomatic dyspnea with irregularities in respiratory rate and depth. They may also cause forced inspiratory spasms, sighing respirations and panting (Calabresi *et al.*, 2010). They tend to occur as a peak-dose phenomenon, appearing after introduction or increase in levodopa medication, and resolving with levodopa dose reduction (Rice *et al.*, 2002). They may be associated with facial and orobuccal dyskinesias (Brown, 1994; Jankovic and Nour, 1986; Rice *et al.*, 2002; Weiner *et al.*, 1978). Reports of respiratory dyskinesia occurring as an "off-period" phenomenon have also been described (Gardner *et al.*, 1987; Mehanna and Jankovic, 2010; Rice *et al.*, 2002).

The incidence of levodopa-induced dyskinesia is clearly related to duration of therapy, however the reported percentages vary greatly in the published literature. There are many factors that may be contributing to this discrepancy including differing patient cohorts affected by various risk factors. In addition, heterogeneity of diagnostic criteria and differences in thresholds for recognizing motor complications are also likely to contribute to this inconsistency in data. It does appear, however, that the occurrence of dyskinesias is higher and of earlier onset than previously thought. In the past, reviews examining mainly retrospective data have estimated median dyskinesia frequencies of less than 10% at 1 year of treatment, increasing to 40% after 5 years of levodopa therapy (Ahlskog and Muenter, 2001). The ELLDOPA trial, a large, randomized trial designed to examine the impact of levodopa on PD progression, however, found that in patients treated with levodopa 600 mg per day, dyskinesias developed in 16% after only 9 months of therapy (Fahn *et al.*, 2004). In addition to duration of treatment, total daily levodopa dose is also a risk factor for the development of dyskinesias. This dose-dependent nature of levodopa-induced dyskinesias was demonstrated in a randomized, double-blind, placebo-controlled trial in which 16.5% of patients treated with 600 mg/day of levodopa for 40 weeks developed LID, in contrast to 2.3% of patients taking 150 mg/day and 3.3% of patients taking 300 mg/day. The addition of the dopamine agonist pramipexole also increased the severity of dyskinesia in patients already on levodopa with pre-existing dyskinesias (Brodsky *et al.*, 2010).

On the other hand, the use of the dopamine agonist ropinirole prolonged-release as adjunctive therapy to levodopa in early PD appeared to delay the onset of dyskinesias in one study, compared to those patients who were treated with increased levodopa (Watts *et al.*, 2010). A long-term observational study suggested that although the frequency of dyskinesia was increased with levodopa use after 5 years, the frequency of dyskinesia was similar after 10 years, regardless of the initial

Parkinson medication used. In this study, 43/64 (67%) patients took levodopa as their initial medication, and by 5 years, all patients were on levodopa (Lopez *et al.*, 2010).

Age has also been shown to be a significant risk factor for the development of levodopa-induced dyskinesias. A number of studies have shown an inverse relationship between the age of PD onset and the incidence of dyskinesias (Jankovic, 2005b; Kumar *et al.*, 2005; Quinn *et al.*, 1987; Schrag *et al.*, 1998; Wagner *et al.*, 1996). There is a higher incidence of choreiform dyskinesia and dystonia in levodopa-treated patients with PD onset before the age of 65 years, compared with the older age group (Wagner *et al.*, 1996). In a retrospective population-based study individuals with PD onset between 40–59 years were found to have a 50% risk of developing dyskinesias at 5 years. This was in contrast to a 26% risk in individuals with onset of PD in the 60–69 years age group, and a 16% risk in those who developed PD after the age of 70 years (Kumar *et al.*, 2005). The incidence of levodopa-induced dyskinesias has been estimated at 91% at 5 years in individuals with PD onset before the age of 40 years (Schrag *et al.*, 1998). The relationship between younger age of onset and increasing dyskinesia risk may not be a linear association, with a significantly higher risk found in one study after 5 years of treatment in patients with PD onset between 40–49 years, compared with those with onset between 50 and 79 years (Ku and Glass, 2010). In this study, 70% of patients with PD onset between 40 and 49 years developed dyskinesia after 5 years of therapy, compared to 42% of patients with PD onset between 50 and 59 years. It was also found that after 5 years of therapy, the risks become similarly high across all ages of onset between 40 and 79 years.

Other possible associations identified in one patient sample included female sex, body weight and Hoehn–Yahr score (Zappia *et al.*, 2005). A number of genetic polymorphisms have been implicated in a few studies, but this has not been a consistent finding. A certain DRD2 polymorphism may increase the risk of dyskinesias in men (Zappia *et al.*, 2005). The DRD2Taq1A polymorphism has also been associated with an increased risk for developing motor fluctuations (Wang *et al.*, 2001). The presence of diphasic dyskinesia, but not peak-dose dyskinesia, has been associated with a polymorphism of DRD3 p.S9G (Lee *et al.*, 2010). In contrast, one study found no association between genetic polymorphisms in DRD2, DRD3 and DRD4 and dyskinesia risk, but instead showed that the 40-bp VNTR of the DAT gene was a predictor for dyskinesias. The met allele of the BDNF gene has been associated with an increased risk of dyskinesia earlier in the course of dopaminergic therapy (Foltynie *et al.*, 2009). Certain monogenic forms of Parkinson's disease may be associated with a higher frequency of dyskinesia. Dyskinesias have been reported to occur in all patients with the parkin (PARK2) mutation (Khan *et al.*, 2003).

Particularly in the early stages, levodopa-induced dyskinesias may be mild, non-persistent, or functionally inconsequential. Dyskinesias requiring medication

adjustment are estimated to affect 17% of patients after 5 years of treatment, and 43% of patients at 10 years in population-based studies.

The impact of levodopa-induced dyskinesias on quality of life is not clearly defined. Although one study of early PD patients suggested that dyskinesias did not cause significant negative impact on quality of life after 4 years of follow-up (Marras *et al.*, 2004), a prospective observational study of patients in various stages of PD found that dyskinesias did adversely affect quality of life, and this remained significant after adjusting for disease progression and motor fluctuations (Péchevis *et al.*, 2005). A multicenter survey found that the level of concern regarding dyskinesias was higher in patients without dyskinesias compared to those who had developed dyskinesias (Hung *et al.*, 2010). In addition, patients with dyskinesias were more likely to prefer better control of their PD symptoms at the expense of having more dyskinesia.

## VIII. Assessment of Dyskinesia

Several rating scales are used to assess severity of dyskinesias. Some of these were created specifically for dyskinesia, however, many have been taken from global scales used for motor assessment in PD (Colosimo *et al.*, 2010). One of the difficulties in creating a universal standardized rating scale has been the inherent variable nature of dyskinesias, which may fluctuate over time and also change with activity. Difficulties are also encountered in assessing the different types of dyskinesias in a standardized scale. Thus objective assessments by an examiner are likely to be restricted to the one point in time during which the evaluation occurs. In contrast, subjective evaluations by the patient are likely to be influenced by individual perceptions of the disease, and also be limited by their ability to differentiate parkinsonian tremor from dyskinesias.

Various rating scales have been evaluated by a task force commissioned by the Movement Disorder Society to evaluate dykinesia in PD (Colosimo *et al.*, 2010). The Abnormal Involuntary Movement Scale (AIMS), which was originally designed to assess tardive dyskinesia and the Rush Dyskinesia Rating Scale (RDRS) are predominantly used to assess dyskinesia in patients with PD, but other scales are also being evaluated for potential use in clinical trials (Colosimo *et al.*, 2010). The original Unified Parkinson Disease Rating Scale (UPDRS) has been modified by a task force of the Movement Disorder Society (MDS-UPDRS) to better capture not only some of non-motor features of PD, but also to better assess levodopa-induced dyskinesias (Goetz *et al.*, 2008). (Further information may be obtained at http://www.movementdisorders.org/publications/rating_scales/).

## IX. Moving Toes and Moving Fingers

A characteristic abnormal involuntary movement involving the toes has been described in the setting of pain, often secondary to peripheral injury (Montagna *et al.*, 1983; Nathan, 1978; Spillane *et al.*, 1971). Pain typically precedes the onset of movement by days to years. The pain is typically very severe, and is most often burning in character (Alvarez *et al.*, 2008). Throbbing, crushing, searing pain, or a deep dull ache may also be described. The distribution of pain is often diffuse, and not limited to an anatomical region (Fahn and Jankovic, 2007g). Complex movements involving various combinations of flexion, extension, abduction, and adduction are seen. The movements may be bilateral or unilateral. When they occur bilaterally, the movements tend to be asynchronous. Surface electrophysiological studies may reveal irregular or semi-continuous EMG bursts. The movements may be partially suppressible. In one series, radiculopathy and neuropathy were found to be the most common pre-disposing factors (Alvarez *et al.*, 2008). Painful legs and moving toes has also been reported in association with central nervous system disorders, with one case occurring in the setting of Hashimoto's encephalopathy (Guimarães *et al.*, 2007).

## X. Myoclonus

Myoclonus is characterized by involuntary brief, jerky, shock-like movements, with preservation of consciousness (Shibasaki, 2002). Positive myoclonus occurs when the movements are caused by brief muscle contraction, whereas negative myoclonus occurs in the setting of sudden cessation of muscle contraction, with accompanying loss of tonic electromyographic activity (Shibasaki and Hallett, 2005). The latter, negative, myoclonus is exemplified by asterixis, typically present in patients with hepatic and other encephalopathies. Myoclonus may be localized to one region of the body, as in focal or segmental myoclonus, or it may be generalized. Multifocal myoclonus occurs when many different parts of the body are affected.

Brief muscle spasms may occur in the setting of other movement disorders, and these should be differentiated from myoclonus. Tics are usually associated with a conscious urge to move and a feeling of relief of tension after the movement. In addition, many tics are suppressible, in contrast to myoclonus. Brief muscle movements in dystonia are often associated with dystonic posturing. Mild chorea may be difficult to distinguish from myoclonus. Sometimes myoclonus is rhythmic and can resemble tremor (Fahn and Jankovic, 2007f).

On the basis of electrophysiological studies, myoclonus can be categorized according to its site of origination into cortical myoclonus, brainstem myoclonus, and spinal myoclonus. Rarely, peripheral myoclonus may arise from peripheral nerves, nerve plexi, and spinal roots. Cortical myoclonus, which arises from the sensorimotor cortex, can present as focal repetitive jerks, termed epilepsia paritalis continua (Hallett *et al.*, 1979). Palatal myoclonus, generalized muscle jerks, and exaggerated startle syndromes (hyperekplexia) may be manifestations of brainstem myoclonus. Spinal myoclonus consists of spinal segmental myoclonus, which is restricted to a few spinal segments, and propriospinal myoclonus, which manifests as generalized axial jerks (Fahn and Jankovic, 2007f).

## XI. Myokymia

Myokymia describes fine quivering, rippling, and undulating contractions of parts of muscle fascicles that is often triggered or exacerbated by stress, sleep deprivation, and caffeine and may persist during sleep. They are caused by hyperexcitability of peripheral nerve motor axons (Gutmann and Gutmann, 2004). Myokymia is differentiated from benign fasciculations by electromyographic features of regular groups of motor unit discharges, especially doublets and triplets, occurring with a regular rhythmic discharge (Denny-Brown and Foley, 1948; Fahn and Jankovic, 2007a). They most commonly occur in facial muscles, particularly around the eyelids, but may also involve other facial muscles and upper limbs as in episodic ataxia type 1.

## XII. Myorhythmia

This term has been used in relation to different movements, and its use has evolved over time. In earlier descriptions, the term myorhythmia was used to refer to rhythmic movements seen in torsion dystonia (Herz, 1944). Subsequently, myorhythmia was used in reference to palatal myoclonus, and other rhythmic myoclonias (Monrad-Krohn and Refsum, 1958). It has since been used to describe a coarse, alternating tremor, which is usually relatively rhythmic and regular but can also vary in rate, rhythm, or amplitude over time (Masucci *et al.*, 1984). The movement is of slow frequency ($<3$ Hz), and can be intermittent or continuous. Myorhythmia may affect an isolated limb, or it may affect multiple body parts. When multiple body parts were involved, it could either be synchronous or

asynchronous. Myorhythmia has been reported in stroke, trauma, nutritional deficiency, phenytoin intoxication, Hashimoto encephalopathy, listerial rhombencephalitis, and Hodgkin's lymphoma (Erickson *et al.*, 2002; Park *et al.*, 2010; Wiener *et al.*, 2003). It has also been described in encephalitis associated with refractory celiac disease (Dimberg *et al.*, 2007).

Oculomasticatory myorhythmia is typically seen in Whipple disease. It refers to slow-moving, repetitive, synchronous and rhythmic contractions in the ocular, facial, and masticatory muscles, and consists of pendular vergence oscillations of the eyes and concurrent contractions of the masticatory muscles (Hausser-Hauw *et al.*, 1988; Schwartz *et al.*, 1986). Isolated facial myorhythmia has been reported rarely (Tan *et al.*, 2007).

## XIII. Stereotypy

Stereotypies are co-ordinated, patterned, repetitive, rhythmic movements (Jankovic, 1994, 2005a). They may be involuntary, or may occur in response to an inner sensory stimulus or unwanted feeling. Stereotypies include both motor and phonic types. They may appear purposeless. Motor stereotypies can include repetitive and sequential finger movements, body rocking, chewing movements, and hand waving. Phonic stereotypies include grunting, moaning, and humming. Stereotypies may be classified as simple, such as foot tapping, or complex, such as sitting down and rising from a chair. They may also be classified according to the body part that is involved.

The etiologies of stereotypies are broad, and may range from physiological (seen in otherwise normal children during development) to pathological causes. Adult-onset continuous stereotypies should suggest the diagnosis of tardive dyskinesia. Stereotypies may also be a feature of a number of neuro-behavioral disorders including frontotemporal dementia, Tourette syndrome, autism, mental retardation, and schizophrenia.

## XIV. Tardive dyskinesia

Tardive dyskinesia refers to involuntary movements that occur as a complication of long-term dopamine receptor antagonist therapy. In a 3-year prospective Schizophrenia Outpatient Health Outcomes study, the incidence of tardive dyskinesia ranged from 2.8% with olanzapine therapy to 11.1% with the use of depot

typical anti-psychotic medication (Novick *et al.*, 2010). The frequency in chronic schizophrenic patients on anti-psychotic medication may be as high as 20–25%. Reported risk factors have included older age, female gender, duration and intensity of antipsychotic treatment, and affective disorders although these are not consistently correlated (Tenback *et al.*, 2009). Other possible associations include first generation antipsychotic use (Correll and Schenk, 2008), ethnicity, and early extrapyramidal symptoms. Genetic pre-disposition may be another risk factor (Greenbaum *et al.*, 2010). The principle site of involvement is classically the oral-buccal-lingual area, and this may be referred to as OBL dyskinesias. The movements are typically repetitive, complex, and coordinated, often resembling normal activity such as chewing. Occasionally, patients with tardive stereotypy may also exhibit lip smacking, lip pursing, sucking, and puckering movements (Fahn and Jankovic, 2007f). Lingual protrusion dystonia may also occur (Esper *et al.*, 2010). Involvement of the forehead and eyebrows is less common. Limb and trunk involvement may also occur in addition to the mouth movements, but these areas tend not to be as severely affected. The toes may exhibit a repetitive flexion and extension movement, as well as foot tapping. The term "piano playing fingers and toes" has been used to describe this characteristic repetitive appearance (Fahn and Jankovic, 2007f). Repetitive movements of the legs such as adducting-abducting of thighs and crossing-uncrossing of legs are commonly seen in patients with tardive dyskinesia and tardive akathisia. Some patients also display rhythmic truncal rocking movements. Other less common involuntary movements seen in tardive dyskinesia include respiratory and esophageal dyskinesias (Horiguchi *et al.*, 1999; Kang *et al.*, 1986; Mehanna and Jankovic, 2010).

The mouth movements of tardive dykinesia typically do not cause significant functional impairment. The movements may temporarily cease during talking, when food is being placed in the mouth, or when a finger is placed on the lips. As a result, patients may not be aware of the presence of these movements. Occasionally, however, patients will report interference with chewing and talking. On examination, the tongue assumes a continuous writhing and coiling movement. In contrast to Huntington disease, motor impersistence is not a feature, and therefore most patients can maintain voluntary sustained tongue protrusion. Many patients with tardive dyskinesia also complain of an uncomfortable sensation in the mouth, tongue, and genital area.

Other movement disorders may also occur as part of a tardive syndrome. Tardive dystonia often resembles idiopathic dystonia. They may improve with sensory tricks. Tardive dystonia can be focal (such as in tardive cervical dystonia and tardive blepharospasm), segmental, or generalized. Similar to idiopathic dystonia, the distribution of tardive dystonia appears to be related to the age of onset (Kang *et al.*, 1986; Kiriakakis *et al.*, 1998). The site of onset ascends rostrally as the age of onset increases. Furthermore, younger onset dystonia is more likely to become generalized. Thus, childhood-onset tardive dystonia tends to start in the lower limbs

and gradually progress to become more widespread, whereas adult-onset dystonia is more likely to remain focal or segmental, and affect the craniocervical region. There are some clinical features, however, that are more characteristic of tardive dystonia. In cervical dystonia, the presence of retrocolis is more common in the tardive variety than in the idiopathic group. In addition, opisthotonic posturing with internal rotation of the arms, elbow extension and wrist flexion can also been seen in tardive dystonia, in contrast to the more lateral truncal twisting in idiopathic dystonia. Finally, whereas voluntary movement often exacerbates idiopathic dystonia, movements such as walking may actually reduce tardive dystonia.

Persistent akathisia (tardive akathisia) may occur in the setting of neuroleptic drug exposure, and may persist despite discontinuation of the medication. The clinical features are believed to be similar to acute akathisia. Moaning and focal pain appear to be more common in tardive akathisia than the acute variety. Although it may exist in isolation, other tardive syndromes, such as dystonia or dyskinesia, often accompany tardive akathisia.

## XV. Tics

Tics are the clinical hallmark of Tourette syndrome (Jankovic, 1997). Both motor tics and phonic tics typically occur in Tourette syndrome. Phonic tics are often considered to be due to motor tics that affect the respiratory, laryngeal, oral, and nasal musculature (Lyon *et al.*, 2010).

Tics are typically sudden, intermittent, repetitive stereotyped movements or phonic productions, and may be involuntary or semi-voluntary. They may appear as fragments of normal action that are misplaced in context (Jankovic and Kurlan, 2011; Leckman *et al.*, 2001). Brief and abrupt tics are referred to as clonic tics. These movements are typically less than 100 ms in duration and jerk-like in character. Examples include blinking, nose twitching, and head jerking. Tics may also be more sustained in nature. Sustained tics are generally more than 300 ms in duration. They can occur in combination with clonic tics in any one individual, and likely represent variants within the same spectrum. Sustained tics can be further classified into dystonic tics and tonic tics. Dystonic tics are associated with twisting, squeezing movements, or posturing. They include blepharospasm, oculogyric movements, bruxism, mouth opening, torticollis, and shoulder rotation. Tonic tics are usually more prolonged, with duration of more than 500 ms. They involve isometric muscle contractions, and may not be associated with movement. Examples include abdominal or limb tensing. The so-called blocking tic, which consists of sudden and transient cessation of motor activity without alteration of consciousness, may be due to prolonged tonic or dystonic tics.

Tics may also be classified as simple or complex. Simple motor tics involve brief jerk-like movements involving only one muscle group. Simple phonic tics can involve brief occurrences of sniffing, throat clearing, grunting, screaming, coughing, blowing, or sucking sounds. Pathological laughter has also been reported as a manifestation of a simple phonic tic (Cavanna *et al.*, 2010). In contrast, complex tics are more coordinated and sequenced. Complex motor tics may resemble normal motor acts or gestures, but are generally inappropriately intense and timed (Jankovic, 1997). The movements can appear purposeful, such as touching, throwing, hitting, jumping, kicking, or non-purposeful, such as head shaking or trunk bending. Occasionally tics can be so severe as to cause neurological sequelae, with reports of compressive cervical myelopathy resulting from recurrent head thrusting and violent neck hyperextension tics (Krauss and Jankovic, 1996). Complex motor tics can also include copropraxia (grabbing or exposing one's genitals) or echopraxia (imitating gestures). Complex phonic tics may consist of linguistically meaningful verbalizations, and can include coprolalia (shouting obscenities or profanities), echolalia (repeating someone else's words or phrases), and palilalia (repeating one's own utterances, particularly the last syllable, word, or phrase in a sentence). Rarely, tics may be continuous and disabling, resulting in so-called "tic status" (Kovacs *et al.*, 2011).

Tics are preceded by premonitory sensations or urges in over 80% of individuals. The urges transiently subside following execution of the tic. Dystonic tics are more likely than clonic tics to be associated with premonitory symptoms (Jankovic, 1997). One study found that, in contrast to most non-tic movement disorders, 68% (40/61) of patients with tics reported that their tics were intentionally produced, whilst a further 25% described both voluntary and involuntary components. Thus, although the majority of movements are perceived as intentional, they are "irresistibly but purposefully executed" (Lang, 1991). Patients may need to repeat a particular movement to relieve the premonitory urge until "it feels just right" (Leckman *et al.*, 1994). It has also been suggested that these patients have an abnormal experience of volition, in which the experience of conscious intention is delayed (Moretto *et al.*, 2011).

Premonitory sensations may be localizable or non-localizable. Localizable sensations include a feeling of tension, tightness, itch, pulling, stretching, burning, pressure, tickle, warmth or other abnormal sensation that is relieved by the tic. They are sometimes referred to as sensory tics (Kurlan *et al.*, 1989). They are primarily localized to the shoulder girdle, palms, midline abdominal region, posterior thighs, feet, and eyes (Jankovic, 1997). A rare phenomenon is that of extracorporeal "phantom" tics, in which the tic is provoked by sensations projected to other people, inanimate or even non-existent objects (Karp and Hallett, 1996), that are temporarily relieved by touching or scratching. Non-localizable sensations, on the other hand, are premonitory phenomena that are

less well-defined and less specific, and include feelings of anxiety, anger, urge, and other psychic sensations (Jankovic, 1997).

There may be some overlap between premonitory sensations, which include feelings of incompleteness and the need for "just-right" sensations, with compulsive behavior (Kwak *et al.*, 2003). These have been referred to as "compulsive tics" (Leckman *et al.*, 1994). Indeed, complex mental events are often experienced by Tourette patients, with tics needing to be performed in a certain manner or a certain number of times in order to satisfy an internal urge (Jankovic and Kurlan, 2011). Tics and compulsive behaviors may often co-exist, and may not always be easily distinguished (Kurlan, 2010). Obsessive-compulsive symptomatology may involve intrusive and disturbing thoughts (obsessions), which in turn lead to ritualized behaviors (compulsions) (Jankovic and Kurlan, 2011). The presence of repetitive behaviors in Tourette syndrome has been attributed to both tic-like behaviors (such as touching and counting), and obsessive compulsive behaviors (such as in checking rituals) (Worbe *et al.*, 2010). In one study, poor quality of life was associated with the comorbid symptoms of depression, obsessive compulsive disorder, and attention deficit disorder, but not tic severity (Eddy *et al.*, 2010).

Another feature of tics is that they may often fluctuate in frequency, intensity, and distribution. They may be transiently suppressible. However, suppression may be associated with a build-up of inner tension, resulting in subsequent release of more forceful tics (Jankovic and Kurlan, 2011). Potential exacerbating factors include stress, excitement, boredom, and fatigue (Jankovic, 1997). In one report, there was a marked exacerbation of tics in response to heat exposure and exercise (Lombroso *et al.*, 1991). The frequency of tics may also increase during a period of relaxation following stress. CNS stimulant medication and dopaminergic drugs may also exacerbate tics. Suggestibility is another feature of tic disorders, and tic frequency may increase when patients are asked about their symptoms. In contrast to many other hyperkinetic movement disorders, tics can persist during sleep.

By definition, the diagnosis of Tourette syndrome involves the onset of tics before the age of 18 years. The so-called adult-onset tic has been reported to represent re-emergence of childhood-onset tics in the majority of cases (Jankovic *et al.*, 2010). In addition, adult-onset tics tend to have troublesome facial and truncal tics. These patients were also found to have an increased risk of depression and substance abuse, compared to those with childhood onset tics.

### XVI. Tremor

Tremor is an oscillatory movement or a rhythmic back-and-forth movement of a body part (Sanger *et al.*, 2010). It is often produced by alternating contractions of

agonist and antagonist muscles. Tremor may be broadly classified as rest tremor, postural tremor, or action tremor. Rest tremor refers to tremor that is present when the affected body part is fully supported against gravity and not actively contracting. The tremor is reduced or disappears with voluntary muscle contraction, or during movement. The typical rest tremor of PD has a frequency of 4–6 Hz, and is most prominent distally. Its characteristic appearance in the hand is also referred to as a pill-rolling tremor. Postural tremor is present with maintenance of a particular posture such as holding the arms outstretched in front of the body. It is commonly seen in physiological and essential tremor. Re-emergent tremor refers to a postural tremor that occurs after a variable latency period during which time no observable postural tremor is present (Jankovic *et al.*, 1999). This typically occurs in the setting of PD, and most likely represents a parkinsonian rest tremor that has been "reset" during maintenance of a posture (Fahn and Jankovic, 2007d) It typically has the same slow frequency as the Parkinson's rest tremor, as opposed to the higher frequencies seen in the postural tremors of physiological and essential tremor. Action tremors occur with voluntary contraction of muscle. The term kinetic tremor may be used to refer to a tremor that occurs during voluntary movement. It includes tremor that occurs with initial movement (initial tremor), as well as that which occurs during active movement (dynamic tremor). Kinetic tremor may also be seen as the affected body part approaches a particular target. An example of this can be seen on finger-to-nose testing, with tremor occurring in the finger as it reaches the nose or the examiner's finger. This type of kinetic tremor is referred to as terminal, or intention, tremor, and is associated with cerebellar dysfunction. Task-specific tremor occurs only during execution of a particular task such as writing. Position-specific tremors are tremors that only occur when the affected body part is placed in a particular position or posture. Orthostatic tremor is an example of a position-specific tremor, and refers to a fast (14 16 Hz) tremor, mainly affecting the trunk and legs, that occurs after standing for a certain period of time (Jankovic, 2009). Isometric tremor occurs during isometric contraction, when muscle is contracted without a change in its length. Dystonic tremor may occur in the setting of dystonia, and may be less rhythmic than other tremor types (Sanger *et al.*, 2010). The appearance may vary depending on the posture of the limb. A "null point" may exist at a particular posture, where there may be minimal tremor.

## XVII. Conclusion

This overview has focused on the clinical spectrum and phenomenology of abnormal involuntary movement disorders, also referred to as dyskinesias. The ability to accurately classify the phenomenology and clinical syndrome of

movement disorders provides crucial information towards determining the possible etiologies, and guides the assessment towards the underlying diagnosis.

TABLE CLINICAL CHARACTERISTICS OF DYSKINESIAS

| Movement Disorder | Key Clinical Features |
|---|---|
| Akathisia | Sensory: feeling of inability to keep still |
| | Motor: stereotypic, complex, restless appearance |
| Athetosis | Slow |
| | Continuous |
| | Writhing |
| Ballism | Forceful, flinging |
| | High-amplitude |
| | Proximal limb |
| Chorea | Non-rhythmic, random |
| | Irregular |
| | Flows randomly from one body part to another |
| | Variable duration and direction |
| Dystonia | Sustained muscle contraction |
| | Twisting or repetitive movements |
| | Abnormal postures |
| Jumpy stumps | Jerking, tremulousness, or spasms |
| | Phantom pains |
| Levodopa-induced dyskinsia | *Peak dose*: Pre-dominant neck, axial, upper limb involvement |
| | Chorea, stereotypies, ballism |
| | *Diphasic*: Starts in legs, "ascending wave" |
| | Stereotypies, large-amplitude, rhythmical |
| | Dystonia, chorea |
| | *Off period*: dystonia |
| Moving toes and moving fingers | Burning pain |
| | Complex, repetitive, rhythmic movements |
| Myoclonus | Brief |
| | Shock-like |
| | Often non-rhythmic |
| Myokymia | Quivering, rippling, and undulating contractions |
| Myorhythmia | Rhythmic |
| | Slow frequency (1–3 Hz) |
| | Intermittent or continuous |
| Stereotypy | Coordinated |
| | Patterned |
| | Repetitive |
| | Rhythmic |
| Tardive dyskinesia | Complex bucco-oral-lingual, lower limbs Stereotypy, chorea |
| | Dystonia |
| | Akathisia |
| | Discomfort (mouth, tongue, genitals) |

(*continued*)

(*continued*)

| Movement Disorder | Key Clinical Features |
|---|---|
| Tics | Sudden, intermittent, repetitive, Stereotyped<br>Premonitory urge<br>Involuntary or semi-voluntary |
| Tremor | Oscillatory<br>Rhythmic back-and-forth movement |

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# L-DOPA-INDUCED DYSKINESIA—CLINICAL PRESENTATION, GENETICS, AND TREATMENT

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Levodopa-induced dyskinesia (LID) has been recognized since the introduction of levodopa for the management of Parkinson's disease (PD) and continues to be one of the most clinically challenging factors in long-term management of patients with PD. Most patients develop LID within 10 years of PD onset and the cause has been attributed to various factors including disease demographics, pharmacological, and possibly genetic causes. The clinical pattern of LID varies and shows intra and inter-patient variability and has been classified based upon phenomenology and relation to timing of levodopa. The potential armamentarium to address and manage LID has significantly increased in the last decade. This chapter addresses the current understanding of various clinical aspects and available therapeutics for LID.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00002-X




0074-7742/10 $35.00

## I. Introduction

The word "dyskinesia" is derived from Greek roots meaning "troubled movements" (*dys*-trouble, *kinesis*- movement). According to Stedman's medical dictionary dyskinesia means: "A*bnormal involuntary movements attributed to pathologic state of one or more parts of the striate body and characterized by insuppressible, stereotyped, automatic movements that cease only during sleep."* Early medical literature on dyskinesia started to appear following the use of neuroleptics in the early 1950s (Wolf *et al.*, 1997) and the first clear description of clinical dyskinesia was recorded by Schoenecker in 1957 (Schoenecker, 1957). The term "tardive" dyskinesia was first coined in 1964 to indicate abnormal movements induced by neuroleptics (Faurbye *et al.*, 1964). Clinically dyskinesia compromises a wide spectrum of motor phenomenology that is fairly similar despite varied etiologies. In Parkinson's disease (PD), dyskinesia was recognized with the advent of levodopa, and since then"levodopa induced dyskinesia"(LID) has become one of the major clinical limitations of long-term management. Despite recent advances and the development of alternative dopaminergic medications for management of PD, dyskinesia still remains a clinical problem with a need to understand the pathophysiology and develop novel therapies. In this chapter we will review the current understanding of epidemiology, clinical phenomenology, genetics, and available therapeutics for dyskinesia in PD.

## II. Historical Aspects

LID was not commented in the initial paper of Cotzias (1967) when describing the successful utility of D,L-dihyroxyphenylalanine. Soon reports of LID started to appear in literature with first publication as early as 1969 (Cotzias *et al.*, 1969; McDowell *et al.*, 1970). Subsequently, all major clinical series on PD started to notethe appearance of LID (Schwarz and Fahn, 1970; Yahr *et al.*, 1969) and by the early 1970s, LID was the most common dose limiting adverse effect (Calne *et al.*, 1971).

By the late 1970s, the clinical phenomenology of LID was being carefully studied and now familiar descriptions were coined for the various terms associated with LID. Thus, dyskinesia noted at the peak clinical benefit of levodopa was called "Peak dose dyskinesia". In 1977, Muenter *et al.* (1977) described dyskinesia appearing at the beginning and at the end of each levodopa dose, which they termed Dystonia-Improvement-Dystonia ("D-I-D") or alternatively labeled as "diphasic dyskinesia" (Marsden *et al.*, 1982). In 1979, Melamed described painful dystonia occurring in the foot early in the morning, when the effect of previous night's

dose of levodopa had completely worn off which was termed "OFF dystonia" (Melamed, 1979). In 1982, Marsden classified dyskinesia based upon two criteria: clinical phenomenology and timing of symptoms in relation to levodopa's clinical effects, which still holds good today (Marsden *et al.*, 1982).

## III. Epidemiology

LID is a well-recognized entity, however not every patient on levodopa develops dyskinesia and even for those who develop LID, the timing of appearance may vary. In addition, assessing the frequency of LID can be difficult as patients frequently have LID but may be unaware of the presence of involuntary movements, unless bothersome. Hence answers to who develops dyskinesia; how commonly they are noticed by patients; and at what time during the disease course they appear may be difficult to accurately ascertain (Marras and Lang, 2003). Thus, there is variability in the frequency of LID reported in the literature since the introduction of levodopa in 1960s. The first major review article on LID was published in 1974, involving 116 patients, where LID was noted within a month of therapy and increased from 20 to 81% by the end of 1 year (Duvoisin, 1974). However, these publications were considered part of the "pre-Levodopa era" in which patients had significant duration of the disease and motor disability (and hence dopamine loss) before the time of initiation of levodopa and thus do not reflect current practice. Thus, more recent literature does not report such a large population of patients being affected so early in the treatment. In a major review of frequency of LID wherein published literature on LID from 1966 to 2000 was reviewed and reported that less than 10% of cumulative patients had LID within 1 year of levodopa therapy and this increased to 25% by 2.5–3.5 years, 35–40% by 4–6 years and reached almost 90% by those who received treatment beyond 9 years (Ahlskog and Muenter, 2001).

Since then, major long-term follow-up publications on therapeutic trials of dopaminergics drugs in PD have replicated similar frequencies of LID. In the 10 year extended follow up of PD patients who were initially randomized to receive levodopa or ropinirole, 77.8% patients who were initially randomized to receive levodopa had developed dyskinesia (Hauser *et al.*, 2007). Long-term follow-up of subjects initially randomized to receive bromocriptine versus levodopa, for example bromocriptine 14 years follow-up and The Sydney multicenter study, noted that among those who survived atleast for 15 years, 94% of patients had developed dyskinesia (Hely *et al.*, 1994; Hely *et al.*, 2005; Katzenschlager *et al.*, 2008). A definitive long-term follow-up of PD subjects

between 1968 and 1996 with clinico-pathological correlation also reported similar outcomes with 61.9% developing dyskinesia after average 9 years of follow-up (Rajput *et al.*, 2002). Thus overall, dyskinesia is a common, almost inevitable, consequence in patients with advanced PD.

## IV. Risk Factors

As noted above, dyskinesia occurs more frequently in advancing disease and corresponding long-term use of levodopa. Thus, the principal causes of LID are a combination of pathological changes and chronic pulsatile dopaminergic receptor stimulation. The early appearance of LID in PD subjects with more advanced disease also confirms the link between disease severity and propensity for LID (Fahn and Bressman, 1984; Montastruc *et al.*, 1999; Weiner, 1999). The main factors associated with the development of dyskinesia are summarized in Table I.

The common risk factor is the disease duration and several studies report that longer disease duration with greater disease severity is associated with an increased risk for LID (PSG, 1996; Rajput *et al.*, 2002; Sharma *et al.*, 2010; Tanner *et al.*, 1985).

The age at onset of PD has also been determined as one of the prominent risk factors for developing LID. Thus, younger age of disease onset is a high risk factor for developing LID (Kostic *et al.*, 1991; Schrag and Quinn, 2000; Sharma *et al.*, 2010; Van Gerpen *et al.*, 2006). It has been noted that about 53% of younger onset patients (onset age 50–59 years) develop dyskinesia at 5 years as compared to 16% with the age of onset at 70–79 years (Kumar *et al.*, 2005). However, these frequencies are less compared to earlier reports of 94% dyskinesia in young onset PD (Quinn *et al.*, 1987). The cumulative dosage of levodopa and longer duration of treatment are also associated with higher risk of developing LID (Miyawaki *et al.*,

Table I
Risk Factors Possibly Associated With Development of Dyskinesia.

1. Early age at onset of PD
2. Longer duration of PD
3. Rate of progression of PD
4. Longer duration of treatment with Levodopa
5. Cumulative dose of Levodopa exposure
6. Severity of PD
7. Female gender
8. Genetic factors including genetic parkinsonism

1997; Tanner *et al.*, 1985). In a community-based study of risk factors for the development of LID and motor fluctuations, shorter time from symptom onset to initiation of levodopa therapy, younger age and duration of levodopa therapy were identified as the main risk factors (Schrag and Quinn, 2000).

Female gender is also a risk factor for higher frequency of LID (Lyons *et al.*, 1998). In a study looking at determinants of peak dose dyskinesia, involving 105 patients, female sex, as well as earlier age of onset of PD, longer duration of treatment and higher dose of levodopa were significant risk factors (Zappia *et al.*, 2005). This association with female gender may relate to smaller body weight and thus overall higher mg/kg dose of levodopa exposure. In a study assessing the relationship between the per kilogram bodyweight levodopa dosage and development of dyskinesia, data from ropinirole versus levodopa study and REAL-PET study were used (Sharma *et al.*, 2008). The authors noted that higher absolute amount of levodopa dosage and the levodopa dosage per kilogram of body weight was significantly associated with the development of LID but not specifically to female sex (Sharma *et al.*, 2008). The association of oestrogen with dyskinesia has also been investigated; however, to date the link remains unclear (Shulman, 2002; Shulman and Bhat, 2006).

## V. Genetics of Dyskinesia

Several studies have assessed genetic factors and individual genetic variations which may predict the development of LID. In general, based upon studies on single nucleotide polymorphisms (SNPs) and targets encoded by genes of interest, the genetic basis of LID has been suggested to be due to genes implicated in genetic parkinsonism *per se*; as well as dopamine and non-dopamine-mediated neurotransmission (Linazasoro, 2005).

The recent developments in understanding the genetics of PD and association of some of these genetic forms of PD with prominent and early dyskinesia have suggested the involvement of genetic factors. Accordingly, PARK-2 (*parkin*), PARK-6 (*pink-1*), and PARK-7 (*DJ-1*) mutations are associated with young-onset PD and frequent appearance of dyskinesia (Dekker *et al.*, 2003). In addition, PARK 8 (LRRK2) parkinsonism has also been linked to a higher risk of developing LID (OR 4.2 compared to age-matched genetically undefined PD subjects) (Nishioka *et al.*, 2010). Altogether, genetic parkinsonism tends to affect individuals at a younger age, often <30 years, known to be a risk factor for developing LID. However, recent evidence suggests that *parkin*-related PD is associated with a delayed-onset of dyskinesia compared to age-matched non-genetic PD subjects, probably due to an overall lower daily levodopa dose (Lohmann *et al.*, 2009). It remains, therefore, unclear if these genetic abnormalities have a direct effect on the

risk of developing LID or if other mechanisms in line with the earlier age at onset and/or levodopa requirements play a role.

There have been various studies investigating genetic associations of dopamine and non-dopamine receptors implicated in basal ganglia function with LID. In this view, polymorphisms of dopamine D2 receptors but not D1 receptors seem to be linked to a reduced risk of developing LID (Oliveri *et al.*, 1999). In another study, the TaqIA polymorphism located in the gene encoding the D2 receptor, but not polymorphisms in the dopamine D3 and D5 receptors, was shown to increase the risk of developing motor fluctuations in PD patients (Wang *et al.*, 2001).

In a study investigating genetic susceptibility factors of diphasic and peak-dose dyskinesia, diphasic dyskinesia was associated with the dopamine 3 receptor p.S9G variant after adjusting for gender, age at PD onset, Hoehn and Yahr stage, and duration of levodopa treatment. Carrying the AA genotype was likely to shorten the onset of diphasic dyskinesia in relation to the duration of levodopa therapy, while the presence of peak dose dyskinesia was not associated with any of the six genetic variants studied (Lee *et al.*, 2011).

Opioid receptors have been implicated in the pathophysiology of LID. Strong *et al.* (2006) looked at whether particular dopamine and opioid receptor polymorphisms were associated with a risk of earlier onset LID. They found carrying the G-allele of the A118G single nucleotide coding region polymorphism of the μ opioid receptor as well as a history of never smoking were independently associated with the increased risk of earlier onset of dyskinesia.

The role of brain derived neurotrophic factor (BDNF), a factor implicated in synaptic plasticity and hence LID, has also been investigated. PD patients with the met allele of BDNF (associated with lower activity dependent secretion of BDNF) were reported to be at significantly higher risk of developing dyskinesia earlier in the course of treatment with dopaminergic agents (Foltynie *et al.*, 2009).

The importance of these genetic factors in the overall risk of developing LID needs further clarification. However, such findings may have future practical implications for helping predict individual PD patients at risk of developing LID.

## VI. Classification

Several classifications of LID have been proposed based upon the type of movements, timing in relation to levodopa dosage, and combinations of the two (Table II). Prior to the early 1980s most of the literature reports were simply descriptions of LID and the variety of associated movements. The first major classification of LID was proposed by Marsden in 1982 (Marsden *et al.*, 1982). In this classification, combination of both, type of movements and timing of

Table II
VARIOUS CLASSIFICATIONS OF LEVODOPA INDUCED DYSKINESIA.

| Author, year | Proposed Classification |
|---|---|
| Marsden *et al.*, 1982 | 1. Peak dose chorea, ballism, dystonia<br>2. Diphasic chorea and dystonia<br>3. "OFF" dystonia<br>4. Myoclonus<br>5. Simultaneous dyskinesia and Parkinsonism |
| Obeso *et al.*, 1989 | 1. "ON" dyskinesia<br>2. Diphasic dyskinesia<br>3. "OFF" period dystonia<br>4. Dyskinesia without benefit<br>5. Dyskinesia-Parkinsonism<br>6. Paroxysmal dyskinesia<br>7. Nocturnal myoclonus |
| Fabbrini *et al.*, 2007 | 1. Typical forms of dyskinesia<br>a. "OFF" period dystonia<br>b. Peak dose dystonia<br>c. Peak dose chorea and ballism<br>2. Less usual forms of dyskinesia<br>a. Respiratory dyskinesia<br>b. Ocular dyskinesia<br>c. Myoclonic dyskinesia<br>3. Movements considered as controversial to be designated as dyskinesia<br>a. Restlessness/hyperactivity<br>b. Akathisia<br>c. Enhanced tremor |

movements were used to classify LID (Table II). In 1989, Obeso further extended this classification and proposed the following types of LID (Obeso *et al.*, 1989):

1. *"ON" dyskinesia*: Involuntary movements that coincide with the period of greatest mobility ("Peak dose") or when present throughout the whole period of adequate motor capability ("Square wave"). These movements are predominantly choreic in nature and predominantly involve neck, axial, and proximal upper limbs.
2. *Diphasic dyskinesia*: Movements that emerge immediately before the levodopa dose turns the patient "ON" and reappear at the end of the therapeutic benefit. This diphasic phenomenon can either be noted simultaneously, both at the onset and wearing off effect of a single dose response, or can appear only during one phase of the cycle, that is at the onset or at the wearing off. These movements tend to be dystonic and painful, and often involve the legs with stereotypic kicking or flexion/extension of the lower leg.

3. *"OFF" period dystonia*: Consists of dystonic postures of the limbs but can also be generalized or affect the cranial musculature during the period when clinical therapeutic benefit of levodopa is not noted. More commonly presents in one foot in the early morning, but might be segmental or generalized, and occur during any "OFF" period.
4. *Dyskinesia without benefit*: Wherein a dose of levodopa produces dyskinetic effects without a parallel clinical benefit.
5. *Dyskinesia–Parkinsonism*: Characterized by one part of the body being dyskinetic and other being parkinsonian.
6. *Paroxysmal or unpredictable dyskinesia*: Part of true "ON–OFF" phenomenon where dyskinetic symptoms occur at any time when the patients mobility state changes or is about to change.
7. *Nocturnal myoclonus*: Reported as special category and particularly noted in leg of patients with levodopa-induced psychosis. It was also suggested that this could be early manifestation of abnormal sleep pattern. (Currently these jerks are part of sleep-related events than of true dyskinetic spectrum).

Fabbrini *et al.* (2007) have included various types of LID and subdivided them into three broad categories based upon the frequency reported in the literature into (1) typical forms, (2) less usual forms, and (3) movements which are considered controversial to be designated as dyskinesia (Table II).

## VII. Clinical Characteristics

The phenomenology of LID is heterogeneous. The most commonly noted movement disorders associated with levodopa therapy are chorea, chorea-athetosis, and dystonia.

### A. Clinical Phenomenology of Various LID

#### 1. *Chorea*

Choreic movements are characterized by involuntary, irregular, purposeless, nonrhythmic, abrupt, rapid, unsustained movements that seems to flow from one part of body to other. These choreic or choreoathetotic movements are the most common forms of LID which are noticed at various stages of LID and are most commonly associated with peak dose dyskinesia. Chorea usually appears first on the side of the body which is predominantly affected and intensity of movements can vary. The severity of chorea varies from very subtle movements which are nonintrusive and may not be recognized by patients, to bothersome movements which interfere with activities of daily living.

2. *Dystonia*

After chorea, dystonia is the most common form of LID, characterized by sustained muscle contractions. The appearance of dystonia as part of LID can be varied from peak dose, beginning/end of dose dystonia to "OFF" dystonia. The dystonia varies in intensity and pattern from involving focal/segmental muscle groups to all limbs—termed "generalized" dystonia. "OFF" period dystonia requires a special mention due to the characteristic presentation and the most common form of dystonia for which a patient seeks relief. These "OFF" period dystonia symptoms are most commonly seen as early morning dystonia, characterized by dystonia affecting one foot or toes, and are most often painful.

3. *Ballism*

Ballism is characterized by very large amplitude choreic movements of the proximal parts of the limbs causing flinging movements. Such movements can be unilateral or bilateral and mostly noted as a part of severe form of choreoathetosis rather than isolated findings.

4. *Myoclonus*

Myoclonus is a sudden brief shock-like involuntary movement which may rarely be seen as part of LID (when other causes of myoclonus have been excluded including other parkinsonian syndromes or drug induced e.g. due to amantadine) and can occur unilateral or bilaterally (Fahn, 2000).

*5. Other Movements*

In addition, other involuntary movements have also been described to be associated with LID. These movements include respiratory dyskinesia(Jankovic and Nour, 1986; Rice *et al.*, 2002), ocular dyskinesia (Linazasoro *et al.*, 2002), restlessness/hyperactivity, akathisia and enhanced tremor. Some of these movements are considered controversial to be designated as dyskinesia (Fabbrini *et al.*, 2007).

## B. Patterns of LID in Relation to the Timing of Levodopa

As discussed above, there have been various studies looking at the pattern of LID in relation to clinical phenomenology. Obeso *et al.* (1989) classified the type of involuntary movements in relation to levodopa's clinical benefits and classified

as: (1) "ON" / Benefit of dose-related movements—chorea, dystonic movements, cranial dystonia, myoclonus; (2) Diphasic—repetitive alternating movements, dystonic postures; and (3) "OFF" period movements—dystonic postures. Luquin *et al.* (1992) studied the pattern of dyskinesia among 168 patients. Ninety-four per cent of patients showed "ON" period dyskinesia's and 18.5% had a diphasic presentation."OFF" period dystonia was noted in 35.7% of patients. They also noticed a mixture of abnormal movements in one part of the body and parkinsonism in another 10 patients (mixed dyskinesia and parkinsonism). In addition, they observed that 50% of patients showed only one type of dyskinesia, 40% had two different types of dyskinesia, and the remaining had more than two types of dyskinesia. With regard to types of movements, they reported that chorea was the most frequent involuntary movement (generalized—22% and segmental—78%), followed by dystonic postures of limbs (37.5%), repetitive movements of the limbs (14.2%), action dystonia (10.7%), cranio-cervical dystonia (8.9%), mixed movement disorders (5.3%), myoclonus and blepharospasm (3.5%), and tics (0.5%). In another study, Marconi *et al.* (1994) conducted a detailed assessment of the clinical characteristics of dyskinesia using video electromyographic recordings in a small group of PD patients ($n = 15$). They noted that dyskinesia started in the foot, usually in the most affected side by the disease and then spread in an ascending wave to the contralateral side, the trunk and upper extremities. The dyskinesia was considered to be dystonic and ballistic at start and became increasingly choreic as they attained the upper limbs, hence describing the variation of movements in relation to the dosing of medications (Marconi *et al.*, 1994).

Overall, the pattern and the type of dyskinetic movements vary in relation to the dosing of levodopa with chorea being the most common peak dose symptom and dystonia being most common during "OFF" period or diphasic dyskinesia. LID usually first appears on the most affected side by PD and commonly begins in foot followed by the involvement of other anatomical structures. However, even in individual patients, the pattern and type of dyskinesia can vary over time and multiple types of LID can be seen in many patients.

## VIII. Treatment

Current treatment options for dyskinesia are limited; only one drug, amantadine, a *N*-methyl-D-aspartic acid (NMDA) receptor antagonist, has met the designation of *efficacious* by the Movement Disorder Society Evidence Based Medical Review (Fox *et al.*, in press). This designation is based on the results of several small clinical studies (da Silva-Junior *et al.*, 2005; Luginger *et al.*, 2000; Metman *et al.*, 1999; Verhagen Metman *et al.*, 1998).

Table III
THERAPEUTIC ARRAY OF TREATMENTS FOR LEVODOPA INDUCED DYSKINESIA: CURRENT AND IN DEVELOPMENT.

1. Amantadine
2. Deep brain stimulation
3. Continuous delivery of apomorphine
4. Continuous delivery of levodopa
5. Antiepileptics: Levetiracetam, Topiramate, Zonisamide
6. Atypical antipsychotics: Clozapine, Olanzapine, Quetiapine
7. 5 $HT_{1A}$ agonist: Sarizotan
8. $mGlu_5$ receptor negative allosteric modulators: AFQ 056, ADX10059
9. NMDA receptor 2B antagonist: Taxoprodil
10. Selective AMPA receptor antagonist: Perampanel
11. Sodium channel inhibitor with MAO-B inhibitor: Safinamide
12. α-2-adrenergic antagonist: Fipamezole, Idazoxan

There have been various drugs tried for the management of LID (Table III). Many of these have failed when tested in larger double-blind placebo-controlled trials. Three major areas of limitation have hampered the development of new treatments. First, the pharmacology of dyskinesia is incompletely understood. Second, well-established clinical outcome measure are lacking, despite the availability of multiple dyskinesia rating scales (Colosimo *et al.*, 2010). The development of a unitary, sensitive, and robust rating scale has been a challenge because of the different types of dyskinesia, their temporal patterns, anatomical distributions, and associated disabilities. Some scales rely on observations by a physician, some by home diaries where the patient records over several days the time spent with dyskinesia, and some combine these efforts. According to the results of a systematic review by a task force of the Movement Disorders Society, the Abnormal Involuntary Movement Scale (AIMS) and the Rush Dyskinesia Rating Scale (RDRS) formally fulfill the criteria for *recommended* (Colosimo *et al.*, 2010), but still have significant limitations. The AIMS, initially developed for the evaluation of tardive dyskinesia in psychiatric patients, has been modified by several authors for its use in PD, but these modifications raise issues with the scale's overall clinimetric properties. The RDRS focuses on disability or the impact of dyskinesia on specific activities of daily living. The newly developed Unified Dyskinesia Rating Scale (UDysRS) combines elements of the AIMS and RDRS into a single measure to cover both impairment and disability (Goetz *et al.*, 2008). It contains a self-assessment by the patient and an examination by the physician. Another scale of high potential future value once further testing is performed is the Parkinson Disease Dyskinesia Scale-26 which is a

patient-based measure for quantifying the impact of dyskinesia on activities of daily living and quality of life (Katzenschlager *et al.*, 2007).

Finally, dyskinesia are highly susceptible to placebo effects, and therefore sample size calculations and power estimates must include an expectation of improvement even with placebo treatment (Goetz *et al.*, 2007). Noteworthy, the placebo effect may vary considerably between different regional sites in multinational clinical trials(Goetz *et al.*, 2007), and the likelihood of placebo assignment may also influence outcome (Lidstone *et al.*, 2010).

### A. Amantadine

Amantadine reduced dyskinesia severity by around 50% on subjective and objective outcome measures compared to placebo in a cross-over study where patients either received amantadine or placebo for 2 weeks (Luginger *et al.*, 2000). In a second cross-over trial, it improved AIMS scores by 60% compared to placebo during an acute intravenous levodopa infusion without worsening PD motor signs (Verhagen Metman *et al.*, 1998). The same patients received another intravenous levodopa infusion 1 year after completion of the first study (Metman *et al.*, 1999). The magnitude of the antidyskinetic effect was similar to the first trial suggesting sustained effects of amantadine on dyskinesia. This observation has been questioned by the results of one study enrolling 40 patients and reporting that the benefit of amantadine lasted only for less than 8 months (Thomas *et al.*, 2004). By contrast, a recent trial confirmed long-lasting antidyskinetic effects of amantadine compared to placebo in PD patients receiving amantadine for a mean duration of 4.8 years (Wolf *et al.*, 2010).

Finally, the effect of acute intravenous amantadine infusion has been tested in a small cross-over study (Del Dotto *et al.*, 2001). Intravenous amantadine infusion reduced modified AIMS scores by 50% compared to placebo, while Unified Parkinson Disease Rating Scale (UPDRS) motor scores were not different between groups.

### B. Deep Brain Stimulation

Several studies have shown antidyskinetic effects of deep brain stimulation (DBS) of the subthalamic nucleus (STN) and the internal pallidum (Deuschl *et al.*, 2006; Krack *et al.*, 1998, 2003; Volkmann *et al.*, 1998). Antidyskinetic properties may be mediated via direct modulation of the activity of the basal ganglia network or an indirect action through a reduction of concomitant dopaminergic treatment. Surgical approaches to LID will be discussed in a specific chapter of this issue.

### C. Continuous Delivery of Apomorphine and Levodopa

Dopaminergic treatment adjustment such as dose fragmentation, smaller single-doses in case of peak-dose dyskinesia, and increased single-doses in patients with biphasic dyskinesia may be helpful in dealing with dyskinesia through a more continuous stimulation of postsynaptic dopamine receptors (Nutt, 2007). Continuous stimulation also underlies the rationale of subcutaneous apomorphine and intraduodenal levodopa infusion.

Continuous subcutaneous apomorphine infusion has been used for many years in PD patients with severe motor fluctuations and dyskinesia despite optimal oral dopaminergic drug treatment. The open-label experience with subcutaneous apomorphine infusion has been reported in several publications (Garcia Ruiz *et al.*, 2008). Besides the reduction in daily "OFF" time, dyskinesia improved by a mean of 36%. More than half of these patients were not suitable for surgical treatment which corresponds to current practice where apomorphine is reserved for patients with severe motor fluctuations who are not suitable or who are waiting for surgery.

One prospective study has assessed the effect of continuous subcutaneous apomorphine infusion on dyskinesia severity (Katzenschlager *et al.*, 2005). In this trial, patients were evaluated with an acute levodopa and apomorphine challenge at baseline and after 6 months of treatment. During the second examination, dyskinesia severity was decreased by 40% suggesting desensitization of postsynaptic receptors and cascades that underlie the development of dyskinesia. Another small prospective trial has compared STN-DBS with continuous subcutaneous apomorphine infusion (Antonini *et al.*, 2010). In this study, 76% of the patients receiving apomorphine dropped out while only 8% stopped DBS. Reasons for drop-out in the apomorphine group were subcutaneous nodules, insufficient control of motor signs, or death during the 5-year follow-up. However, this study was limited by its open-label design and the lack of randomization.

Beyond classical systemic adverse events due to its action on peripheral and central dopamine receptors, local side effects of continuous subcutaneous apomorphine treatment are common, ranging from pruritic erythema to painful nodules (Garcia Ruiz *et al.*, 2008). Histological examination of skin nodules in 10 patients revealed a florid panniculitis with some fat necrosis and an eosinophilic infiltrate in most patients (Acland *et al.*, 1998). Cutaneous side effects are usually mild to moderate, but require sometimes treatment discontinuation. A dilution of 5 mg/mL apomorphine instead of 10 mg/m and a regular change of the injection site allow reducing local side effects. Ultrasonic treatment may also provide some relief (Hughes *et al.*, 1993; Poltawski *et al.*, 2009).

Neuropsychiatric symptoms are frequent in later disease stages at the time PD patients receive continuous subcutaneous apomorphine infusion. It may therefore be difficult to distinguish between disease-associated symptoms and treatment-related side effects. In a large retrospective series of 82 patients, 26% had hallucinations and 33% cognitive impairment at treatment initiation (Garcia Ruiz *et al.*, 2008). No change was observed during the mean follow-up of 20 months but nine patients were excluded from the analysis because of drop-out in relation to psychosis. In another cohort of 25 patients, three stopped apomorphine because of psychiatric side effects. Overall, 44% showed psychiatric changes during a mean follow-up of 4.5 years, most of them developing concomitant cognitive impairment (Pietz *et al.*, 1998). These results suggest that psychiatric side effects of apomorphine should be thoroughly monitored in patients with cognitive impairment while treatment discontinuation in relation to these adverse events only occurs in a low number of patients.

Continuous nasoduodenal levodopa infusion has been assessed in PD patients with motor fluctuations and dyskinesias in two small randomized controlled trials with a cross-over design (Kurth *et al.*, 1993; Nyholm *et al.*, 2005). In the first study, the efficacy of continuous duodenal levodopa infusion was compared with intermittent oral levodopa in 10 PD patients with motor fluctuations. The clinical outcome was assessed with the Parkinson Mobility Scale, a 9-point scale ranging from "severe abnormal involuntary movements" to "severe slowness" (Kurth *et al.*, 1993). Continuous duodenal levodopa infusion increased total hours of "good" function and decreased plasma levodopa level variability by 47%. All enrolled patients decided to pursue duodenal levodopa infusion after the end of the study. The second study found for continuous duodenal levodopa infusion a 14% increase in "ON" time by reducing "OFF" time without increasing "ON" time with dyskinesias compared to conventional levodopa treatment. Median total UPDRS scores were 53 versus 35 in favor of continuous duodenal levodopa infusion. Quality of life was also significantly improved with levodopa infusion, while safety was not different between treatments.

The experience in daily practice with continuous intraduodenal levodopa infusion has recently been reported for 102 patients (Devos, 2009). In almost all of these patients, intraduodenal levodopa infusion was the last line treatment for motor complications. Accordingly, in 98% of them continuous apomorphine infusion or DBS had failed or were contra-indicated. Dyskinesias were improved in 95% on a 3-point rating scale (improvement - no change - worsening). Most side effects were related to the gastrostomy or the infusion technique (problems with the inner tubing or pump failure) and required discontinuation in 8%. Hallucinations were seen in two thirds and half of the patients were demented. Severe hallucinations were noted in 42% at the beginning of intraduodenal levodopa infusion without any worsening over time.

## D. Re-Positioning of Existing Drugs for the Treatment of Dyskinesia

### 1. *Antiepileptics*

The antidyskinetic properties of different antiepileptic drugs have been assessed in small pilot studies. Accordingly, levetiracetam increased "ON" time without or with non-troublesome dyskinesia by 18% while "ON" time with troublesome dyskinesia was reduced by 12% (Zesiewicz *et al.*, 2005). However, there was a considerable drop-out rate of 56% of patients, mostly because of somnolence. In a second study with levetiracetam, there was also a remarkable drop-out rate (44%) due to worsening of PD symptoms and somnolence (Lyons and Pahwa, 2006). Of the remaining five patients, four discontinued levetiracetam after the end of the study because of side effects. In contrast, a recent exploratory randomized controlled trial (RCT) reported good tolerability and mild significant effect on "ON" time without dyskinesia with levetiracetam 500–1000 mg/d (Stathis *et al.*, 2011). A second RCT showed no significant change in AIMS but mild positive effect on UPDRS IV dyskinesia using doses up to 2000 mg/d, with no major tolerability issues (Stathis *et al.*, 2011; Wolz *et al.*, 2010).

The effect of topiramate on dyskinesia, another approved antiepileptic drug, has been evaluated in several small studies (no results yet reported).

Zonisamide (25–100 mg) has been tested in a large, randomized study including 347 PD patients with motor fluctuations (Murata *et al.*, 2007). The secondary endpoints included UPDRS part IV scores. Patients receiving zonisamide had significantly lower UPDRS motor scores and daily "OFF" time compared to placebo. UPDRS part IV scores were not different between groups, while zonisamide (50 mg) decreased disabling dyskinesia when separately analyzing item 33 of the UPDRS part IV (severity of dyskinesia ranging from 0 = not disabling to 4 = completely disabled). Patients receiving zonisamide complained dose-dependently about more dizziness, apathy and, a decrease in body weight. Zonisa-mide is approved in Japan as a treatment for PD motor symptoms since 2009.

### 2. *Atypical Antipsychotics*

The efficacy of clozapine, a dopamine receptor antagonist with serotonergic, muscarinic, adrenergic and histaminergic action, has been evaluated in several small pilot studies (Bennett *et al.*, 1993, 1994; Durif *et al.*, 1997; Pierelli *et al.*, 1998) and in one larger RCT (Durif *et al.*, 2004). In the latter, 50 dyskinetic PD patients either received clozapine up to 75 mg/d or placebo for 10 weeks. Patients under clozapine gained 2.4 h of "ON" time without dyskinesia compared to placebo, while the duration of "OFF" periods remained unchanged in both groups. Dyskinesia ratings at rest during an acute levodopa challenge were also decreased

in the clozapine group, while dyskinesia severity in the same condition during an activation task was not different. Clozapine had no effect on the antiparkinsonian action of levodopa.

Olanzapine has shown antidyskinetic properties in a small randomized, placebo-controlled cross-over trial. Nine PD patients with physically disabling or socially embarrassing dyskinesia either received olanzapine up to 7.5 mg/d or placebo for 2 weeks (Manson *et al.*, 2000b). Objective and subjective efficacy measures showed a decrease in dyskinesia severity and duration in favor of olanzapine. However, adverse events were more common with olanzapine, consisting in increased "OFF" time, parkinsonism, and drowsiness. As a result, olanzapine is not recommended for use in PD.

Quetiapine, another atypical antipsychotic with few extrapyramidal side effects, has been tested in a small randomized, placebo-controlled, cross-over study (Katzenschlager *et al.*, 2004). Patients either received 25 mg/d of quetiapine or placebo for 2 weeks. No differences were observed between quetiapine and placebo. The double blind trial was followed by an open-label period of around 30 days during which patients received up to 50 mg/d of quetiapine. Mild improvement in dyskinesia duration and severity were observed during the open-label period according to patient home diaries.

### E. Other

Cannabis has been examined in a randomized, placebo-controlled cross-over trial (Carroll *et al.*, 2004). From 59 screened patients, 20 were found to be unsuitable for the study and 14 declined participation. Patients received cannabis extract (standardized to 2.5 mg $\Delta^9$-THC and 1.25 mg cannabidiol) up to a maximum of 0.25 mg/kg THC per day or placebo. Cannabis tended to worsen dyskinesia on UPDRS part IV ratings. No serious adverse events were observed.

### F. New Agents and Targets for The Treatment of Dyskinesia

Research of recent years has focused on the development of non-dopaminergic drugs including molecules acting on serotonin, glutamate, and adrenergic receptors.

Agonists of 5-$HT_{1A}$ and 5-$HT_{1B}$ as well as antagonists of 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors are in development for motor fluctuations and dyskinesia. The most advanced compound is the 5-$HT_{1A}$ agonist sarizotan which failed to demonstrate effective for dyskinesia in two late stage trials (Goetz *et al.*, 2007). Reasons may have included prominent placebo effect (Goetz *et al.*, 2007) and dose limitations due to

lower potency dopamine D2 receptor antagonism. The current status of late development for sarizotan is uncertain at present.

Of all other targets, $mGlu_5$ receptor negative allosteric modulators (NAM) are most advanced in clinical development for dyskinesia. In this view, AFQ056 has shown antidyskinetic effects in parkinsonian non-human primates and in a phase Ib/II clinical trial in PD patients (Berg *et al.*, 2010; Grégoire *et al.*, 2011). AFQ056 decreased AIMS scores by 50% compared to placebo. UPDRS motor scores were also improved by more than three points versus placebo, but the study was underpowered to reach statistical significance. A large RCT is ongoing and ADX10059, another $mGlu_5$ NAM is entering early stage assessments for dyskinesias.

The selective NMDA receptor 2B (NR2B) antagonist taxoprodil has shown antidyskinetic properties while abnormal thinking, depersonalization, and amnesia were frequent side effects (Nutt *et al.*, 2008). Selective AMPA receptor antagonists such as perampanel have also been evaluated in PD patients. However, the drug failed to demonstrate a significant effect on wearing-off, dyskinesia, and cognition (Eggert *et al.*, 2010).

Safinamide, a sodium channel inhibitor with MAO-B inhibitory activity initially developed as an antiepileptic, has been shown to inhibit glutamate release (Schapira, 2010). Safinamide is currently in late stage RCTs for motor control, and is also being explored for potential efficacy against dyskinesia and cognitive impairment in PD.

The effect of fipamezole, an α-2-adrenergic antagonist, on dyskinesia has been assessed in a phase II trial (Lewitt *et al.*, 2010). The overall results displayed no differences between drug and placebo probably because of an important heterogeneity between the enrolling centers. When only looking at US subjects, fipamezole showed a significant decrease in dyskinesia ratings without worsening parkinsonism (Lewitt *et al.*, 2010). Another α-2-adrenergic antagonist, idazoxan, has been tested in two small clinical studies. Results were conflicting (Manson *et al.*, 2000a; Rascol *et al.*, 2001) and the development of this drug for dyskinesia was finally stopped.

Novel approaches to dyskinesia therapy will be more detailed in a specific chapter of this issue.

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# EXPERIMENTAL MODELS OF L-DOPA-INDUCED DYSKINESIA

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Strategies to avoid or minimize dyskinesia and other motor complications of chronic dopamine replacement therapy in Parkinson's disease (PD) remain a significant unmet clinical need. As such, the refinement and development of animal models with which to delineate the underlying molecular mechanisms of dyskinesia and to find effective treatment paradigms remain as necessary as ever. Toxin-based models including the MPTP-lesioned primate and the 6-hydroxydopamine (6-OHDA) lesioned rodent continue to form the bedrock of current L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia modeling approaches. This chapter reviews these models, illustrating their origins, application and strengths as well as problems that accompany their use. We also describe new methodologies that, although still in their infancy, may offer powerful future alternatives by which to better model this debilitating complication of current PD treatment.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00003-1




0074-7742/10 $35.00

## I. Historical Development of a Model of L-DOPA-Induced Dyskinesia

Not long after George Cotzias and colleagues began chronically administering the dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) to patients with Parkinson's disease (PD) in the 1960s (Cotzias *et al.*, 1967) came the recognition that this break-through symptomatic treatment for PD was marred by the development of treatment-related motor complications (Cotzias *et al.*, 1968). Such complications include a shortening in duration of the anti-parkinsonian benefit of L-DOPA ("wearing-OFF"), sporadic benefit ("ON–OFF" phenomenon) and L-DOPA-induced dyskinesia (LID) (Stocchi *et al.*, 2008). These latter abnormal involuntary movements (AIMs) may either be predominantly choreiform or dystonic in nature (Fabbrini *et al.*, 2007) and whilst early L-DOPA treatment regimens often involved what are now recognized to have been profound over-medication of PD patients, LID continues to exert a major negative impact to patient's quality of life (Hung *et al.*, 2010). After 15 years of dopaminergic therapy, LID will affect 95% of PD patients causing significant disability and restricting the use of L-DOPA to combat parkinsonian symptoms (Hely *et al.*, 2005). Fifty years on there is still a limited understanding of LID and a demonstrable need for good animal models. Ideally such a model will recapitulate all the elements of LID although as yet the relationship of LID to different features of the disease is uncertain. It would therefore ideally mimic underlying disease pathogenesis and its behavioral consequences, as well as both the positive and negative effects of L-DOPA treatment. Experimental models of LID have developed out of those used to study the consequences of dopamine denervation and potential therapeutics for PD. While we have made significant progress using this approach they also have significant limitations.

At around the time when treatment-related motor complications such as LID were first being noted in PD patients, the animal models available for PD were centered around use of the rodent or rabbit. The acute administration of the irreversible vesicular monoamine transporter inhibitor reserpine produced a transient, bilateral nonspecific monoamine depletion resulting in profound akinesia, but lacked any PD-like neuronal degeneration (Carlsson *et al.*, 1957). A significant advance came with the discovery of selective neurotoxins such as 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968). This catecholaminergic toxin is taken up by monoamine reuptake transporters, leading to increased oxidative stress and subsequent cell death. At the time, this model provided the closest approximation not only of behavioral symptoms of untreated PD but for the first time true neuronal degeneration. Whilst L-DOPA exhibits anti-parkinsonian efficacy in both the reserpine and 6-OHDA rodent models (Carlsson *et al.*, 1957; Uretsky and Schoenfeld, 1971) it was believed that neither species reproduced the diffuse and complex range of abnormal movements observed in patients with LID. Key in the timeline of dyskinesia research was in 1983 when a contaminant in the

synthesis of meperidine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was identified as the cause of sudden onset parkinsonism in a group of drug addicts (Langston *et al.*, 1983). Not only were their symptoms indistinguishable from idiopathic PD, L-DOPA or dopamine agonist administration also evoked all of the treatment-related motor complications including dyskinesia (Langston and Ballard, 1984). Subsequent administration of MPTP to various species of non-human primate was able to recapitulate all these key elements of parkinsonism including loss of nigrostriatal dopaminergic cells and behavioral sequelae (Burns *et al.*, 1983; Jenner *et al.*, 1984; Langston *et al.*, 1984). Moreover, there was now a model in which chronic administration of L-DOPA provided the closest behavioral correlate of dyskinesia in PD patients yet seen (Bedard *et al.*, 1986; Clarke *et al.*, 1987). This development provided significant advancement in the understanding of the pathophysiology and pharmacology of LID and it continues to be the most robust and clinically relevant model. Over a decade later, Cenci and colleagues published the first paper reasserting a pivotal role for the 6-OHDA lesioned rat model in dyskinesia studies (Cenci *et al.*, 1998). This has since been extended into the mouse (Lundblad *et al.*, 2004), which is now being further advanced with the evolution of transgenic species (Darmopil *et al.*, 2009). This review revisits these models, exploring their role specifically in the development of novel treatments for dyskinesia and critically evaluates the contribution of each, whilst exploring how we can move the field forwards.

## II. MPTP-Lesioned Primate Model of L-DOPA-Induced Dyskinesia

### A. Introduction

After more than 25 years the L-DOPA-treated, MPTP-lesioned primate remains the gold standard in terms of modeling dyskinesia in PD. No current approach surpasses the fidelity with which the key behavioral phenomenology and underlying neurophysiological changes seen in PD patients with LID are reproduced. The MPTP-lesioned primate has been used frequently in the development of novel therapeutics predicting the efficacy and therapeutic outcome of novel dopaminergic (Jenner, 2009) and non-dopaminergic treatments (Fox *et al.*, 2006), surgical (Aziz *et al.*, 1991), and transplantation approaches (Bakay and Herring, 1989). The model has also been pivotal in helping delineate some of the mechanistic underpinnings of LID such as the role of the direct striatonigral and indirect striatopallidal pathways and subthalamic nucleus (STN) in regulating output regions of the basal ganglia in generating the motor symptoms of PD and LID (Crossman *et al.*, 1985; DeLong *et al.*, 1985; Wichmann and DeLong, 2003).

## B. Basis for the Model: the MPTP-Lesioned Primate

Prior to the induction of clinically relevant dyskinesia in any primate species, a parkinsonian phenotype must first be established. Administration of MPTP evokes the primary parkinsonian motor abnormalities seen in PD patients including bradykinesia, postural instability, and rigidity (Burns *et al.*, 1983). Of these, the impact of a potential novel therapy on bradykinesia or akinesia, along with the animal's general range of movement (extent of spontaneous exploratory behavior) is often of utmost importance. A host of primate species have been utilized in studies of parkinsonism and LID with the rhesus and cynomolgus macaque (*macaca mulatta* and *macaca fascicularis* respectively) species being the most frequently employed (Burns *et al.*, 1983). Administration of MPTP to other species including the common marmoset (*Callithrix jacchus*) (Jenner *et al.*, 1984), squirrel monkey (Langston *et al.*, 1984), and African green monkey (Elsworth *et al.*, 1987) has also been successfully undertaken. MPTP is most commonly administered repeatedly via systemic intravenous or subcutaneous injection to give a bilateral parkinsonian syndrome. Although with sufficiently high doses of L-DOPA dysregulation of striatal function and subsequent LID may be invoked in normal intact animals (Pearce *et al.*, 2001), typically the severity of dyskinesia in non-human primates, as with human PD patients is correlated to the severity of underlying parkinsonism (Obeso *et al.*, 2000; Schneider *et al.*, 2003). Thus, most MPTP dosing regimens designed with dyskinesia studies in mind strive to achieve a substantial and irreversible loss of dopaminergic phenotype. By contrast, mild MPTP regimens, for example in the marmoset using low-dose MPTP (1 mg/kg daily for 3 days) to model earlier disease stages, evoked only a 60% loss of tyrosine hydroxylase positive cells in the substantia nigra with animals displaying modest parkinsonian symptoms and a poor response to L-DOPA (Iravani *et al.*, 2005). Furthermore, most primate species exhibit some capacity for spontaneous behavioral recovery if the MPTP regimen employed is not sufficiently robust. Indeed, this feature has been usefully exploited in order to explore potential compensatory mechanisms in early stages of PD (Boulet *et al.*, 2008; Mounayar *et al.*, 2007). In the marmoset a more severe parkinsonian phenotype may be achieved using higher doses of MPTP for longer periods (for example 2 mg/kg once daily for 5 days) (Iravani *et al.*, 2005; Visanji *et al.*, 2009a). In contrast, macaques appear to demonstrate greater variation in their sensitivity to MPTP and most regimens, either higher doses (1–2 mg/kg) given once weekly (Samadi *et al.*, 2003) or lower doses (0.2 mg/kg) given daily for up to 2 weeks (Bezard *et al.*, 1997) may require individual titration of MPTP dose over a period of several months in order to produce a group of animal with comparable levels of disability. A single intracarotid infusion of MPTP has been employed as a means of obtaining a unilateral parkinsonian phenotype akin to that of the 6-OHDA lesioned rat (Bankiewicz *et al.*, 1986). This approach has also been recently extended to combine sequential intracarotid and

systemic MPTP administration to give an asymmetric lesion that incorporates severe and partially lesioned hemispheres (Kells *et al.*, 2010). However, whilst of great utility for studies in which an internal contralateral "control" brain hemisphere remains against which to compare the effect of MPTP-lesion or unilateral therapy the standard intracarotid model may not be best suited for use in dyskinesia studies (Lieu *et al.*, 2011).

In generating more robust MPTP-induced lesions suitable for LID experiments primates of either sex may successfully be employed (Johnston *et al.*, 2010c). Sensitivity to the effects of MPTP becomes of greater significance however when the age of the animal is taken into consideration. For example, young macaques (5–9 years old) required on average 3-fold higher total doses of MPTP to achieve the same level of parkinsonian symptoms as old (20–23 years) animals (Ovadia *et al.*, 1995). Care should therefore be taken in the design of MPTP-primate studies to employ as closely aged a group of animals as possible.

### C. Generation of L-DOPA-Induced Dyskinesia in the Primate

Chronic treatment of PD patients with L-DOPA results in the development of a spectrum of motor complications including both choreiform and dystonic dyskinesias (Fabbrini *et al.*, 2007) that are virtually indistinguishable from those observed in MPTP-lesioned primates (Clarke *et al.*, 1987; Jenner, 2003). Studies in which the ability of a novel therapeutic to impact on LID is assessed commonly fall into two broad categories. Thus, two or more groups of "*de novo*" parkinsonian animals may be treated with L-DOPA either alone or in combination with test compound and the effect on the genesis and development of LID assessed over time (Visanji *et al.*, 2009a). Alternatively, animals are treated chronically with L-DOPA for several weeks or months to evoke established, stable expression of LID prior to assessment of the acute or chronic effect of test compound when co-administered with L-DOPA (Bezard *et al.*, 2004; Henry *et al.*, 1999; Iravani *et al.*, 2003; Johnston *et al.*, 2010b). For each of these studies the propensity of a potential therapeutic to influence the expression of dyskinesia may be tested in various ways. Thus, an agent may act to reduce dyskinesia evoked by optimal or supraoptimal doses of L-DOPA that maximally reverse parkinsonian symptoms (Bezard *et al.*, 2004; Johnston *et al.*, 2010b; Morin *et al.*, 2010). Alternatively, the ability of a therapeutic to enhance the anti-parkinsonian benefit afforded by threshold doses of L-DOPA that only partially alleviate symptoms whilst evoking less dyskinesia than that achieved by merely increasing L-DOPA dose (an L-DOPA "sparing" effect) may also be assessed (Johnston *et al.*, 2010c; Kanda *et al.*, 2000).

The route and dosing paradigm by which L-DOPA is administered in order to elicit dyskinesia is critical. In the macaque, it is often necessary to individually titrate L-DOPA doses in order to elicit the same levels of dyskinesia (Johnston *et al.*,

2010b) in spite of the underlying dopaminergic lesion being comparable in such animals (Guigoni *et al.*, 2005). For primate dyskinesia studies in which the aim is to inform the design and potential outcome of clinical Phase II studies, comparable means of evoking dyskinesia between primate and PD patients should be employed (Fox *et al.*, 2006). Thus, L-DOPA may be given orally at doses that would be comparable to those used clinically but at the risk of insufficient dyskinesia being evoked to demonstrate statistically significant effects of treatment. Conversely, a dose of L-DOPA that is too high, whilst providing robust expression of dyskinesia in the primate, may be such that subtle effects of treatment are missed (Blanchet *et al.*, 1999). Although intravenous infusion of L-DOPA is sometimes used in a clinical proof-of-concept setting in PD patients to allow controlled assessment of dyskinesia with optimal anti-parkinsonian effect (Verhagen Metman *et al.*, 1998a) it is not used in primate models due to the complexities of restraining the animal. Intraperitoneal or subcutaneous routes of L-DOPA administration using the methyl-ester form to provide more stable and reproducible plasma levels are however used commonly (Gomez-Ramirez *et al.*, 2006) thus avoiding potential variability in absorption and first-pass metabolism of L-DOPA associated with the oral route (Cooper *et al.*, 1984).

Differences in the phenomenology of the dyskinetic response to L-DOPA are apparent between various primates with some correlation between phylogenetic complexity of the species and the breadth of PD-like dyskinesias expressed. For instance, macaques readily express both choreiform and dystonic forms of dyskinesia (Boyce *et al.*, 1990a) whereas in the marmoset distinguishing the two types may be less straightforward (Fox and Brotchie, 2010). However, the locomotor activity response to L-DOPA challenge is generally more robust in the marmoset making it an excellent model in which to assess the anti-parkinsonian effect of monotherapy or that of an adjunct treatment in combination with L-DOPA (Fox and Brotchie, 2010).

In all primate species, the severity of dyskinesia generally increases with the duration of frequency of L-DOPA therapy (Kuoppamaki *et al.*, 2007). Much work has focused on the pulsatility of central dopamine receptor stimulation as being key in evoking dyskinesia in both primate and human (Olanow *et al.*, 2006; Smith *et al.*, 2003). Such observations have led to the development of therapeutic strategies to deliver continuous dopamine stimulation with agents such as entacapone (Smith *et al.*, 2005) or transdermal delivery of dopamine agonists (Stockwell *et al.*, 2009). However, in the context of primate modeling, pulsatile dopamine receptor stimulation is often employed to better evoke robust expression of dyskinesia in naive MPTP-lesioned animals (Visanji *et al.*, 2009a). Chronic treatment with L-DOPA in both PD patients and parkinsonian primates not only increases the severity of dyskinesia but modulates its temporal pattern of expression following acute challenge. Thus, upon first administration of L-DOPA to *de novo* animals there is a graded reversal of parkinsonism and accompanying expression of

dyskinesia as a product of increasing L-DOPA dose. In chronically treated animals or in PD patients following long-term therapy, there is a more rapid anti-parkinsonian response (Nutt *et al.*, 2002) and an "all-or-nothing" dyskinetic effect in which increasing the dose of L-DOPA further fails to evoke more any more severe a response (Mestre *et al.*, 2010). The MPTP-primate also successfully models the lack of separation of anti-parkinsonian and pro-dyskinetic properties of L-DOPA seen in chronically treated PD patients (Nutt *et al.*, 2010). Following chronic dosing with L-DOPA in MPTP-primates, usually after several weeks or months of administration, dyskinesia is typically stable and reproducible between dosing (Pearce *et al.*, 1995) thus allowing the reliable assessment of adjunct treatment on acute expression of LID (Johnston *et al.*, 2010e). Peak levels of dyskinesia ("peak-dose") as expressed in parkinsonian primates, typically occurs when plasma levels L-DOPA are maximal (Clarke *et al.*, 1987; Crossman *et al.*, 1987). "Diphasic" dyskinesia may be experienced by PD patients as plasma levels of L-DOPA are rising and falling but is less commonly examined in the MPTP-primate (Boyce *et al.*, 1990b). Interestingly, the expression of diphasic dyskinesia seems temporally correlated with another complication of long-term L-DOPA treatment that of "beginning and end-of-dose worsening." Thus, after acute L-DOPA challenge, animals exhibit a worsening of motor function before any improvement, and at the end of dose period, after decline in L-DOPA benefit, motor performance shows rebound worsening (Kuoppamaki *et al.*, 2002).

### D. Rating Scales

In addition to the assessment of motor complications such as dyskinesia in the L-DOPA-treated MPTP-primate, it is essential that measurements of parkinsonian disability are first conducted. Not only is it vital to describe the extent to which a given dose of L-DOPA affords benefit but also to gauge the level of disability exhibited by the animal in the untreated parkinsonian state. Thus, the therapeutic potential of a treatment that reduces LID is greatly compromised if it also negatively impacts on anti-parkinsonian benefit. A multitude of scales for assessing parkinsonian disability in the primate have been developed (reviewed by Imbert *et al.*, 2000). All the scales currently in use derive from clinically employed rating scales such as the Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz *et al.*, 2008b). Although weighted differently between scales, the cardinal features of parkinsonism including range of movement, bradykinesia, posture, and alertness are all represented. Since the two major primate species employed in PD research, the marmoset and macaque, do not show appreciable levels of tremor in response to MPTP (Fox and Brotchie, 2010), some versions of the scale do not include this parameter (Johnston *et al.*, 2010b). These scales are applied to recorded footage of the animal by trained personnel, optimally neurologists specializing in movement disorders who are blinded to the experimental condition. Progress is

being made in digital computer-based assessment of video-footage (Saiki *et al.*, 2010) although the sophistication required to distinguish between complex movements, for example hyperkinesia and chorea, may still be lacking. Automated measures of total locomotor activity are also employed for instance using passive infrared sensor placed above individual observation cages (Visanji *et al.*, 2009b) or, as a measure of an animal's total activity across whole 24 h periods, accelerometers may be attached to the animal's collar (Johnston *et al.*, 2010d). Assessment of an animal's fine motor skill as well as aspects of motivation and cognitive ability that are impacted upon by MPTP-lesioning and restored by L-DOPA treatment may be made using the monkey Movement Assessment Panel (mMAP) (Gash *et al.*, 1999).

As with parkinsonian disability, scales for the assessment of dyskinesia in primates have in large part been modified from those used to rate dyskinesias in human PD patients (reviewed by Colosimo *et al.*, 2010). Increasingly recognized is the need for methods of assessment in primate to inform the type of endpoints that would be measured at Phase II in the clinic and furthermore bear some comparison to quality of life measures reported by the patient themselves at Phase III (Fox *et al.*, 2006). To these ends, of the existing dyskinesia scales used in the clinic, the Abnormal Involuntary Movement Scale (AIMS) (Guy, 1976) and the Rush Dyskinesia Rating Scale (Goetz *et al.*, 1994) are recommended since both are in regular use and have had multiple clinimetric studies establish that they are valid, reliable, and sensitive (Colosimo *et al.*, 2010). The AIMS scale assesses dyskinesia intensity according to a 4-point scale across seven body parts while the Rush scale assesses the disability imparted by dyskinesia on performance of specific activities. Phase II relevant equivalents of both scales exist in the form of the macaque AIMS scale (Blanchet *et al.*, 1998), marmoset dyskinesia-disability scale (Pearce *et al.*, 1995), and the global non-human primate dyskinesia rating scale (GPDRS) for the squirrel monkey (Petzinger *et al.*, 2001). A recently devised human dyskinesia scale, the Unified Dyskinesia Rating Scale (UDysRS) was developed specifically for the assessment of dyskinesia in PD (Goetz *et al.*, 2008a). Although currently lacking long-term validation across clinical centers, the UDysRS represents a more comprehensive rating system that seeks to capture not only measures of disability and severity of dyskinesia as assessed clinically but also patient perceptions of their dyskinesia, a factor much more relevant to successful outcome at Phase III. As a consequence of this, concerns have arisen that existing primate scales, although generally reliable indicators of efficacy for anti-dyskinetic actions of non-dopaminergic drugs at Phase II, may not adequately serve in the successful translation of potential therapies for motor complications to Phase III trial (Fox *et al.*, 2006; Linazasoro, 2004). This concern reflects the frequent absence of Phase III-relevant endpoints in primate studies. Alternative assessments have been designed to model clinical measures of quality of a treatment's benefit. For example, to provide some measure of proportion of time for which dyskinesia is present (MDS-UPDRS item 4.1) (Goetz *et al.*, 2008b) and diary measures of duration of anti-parkinsonian

benefit (ON-time) that incorporate the impact of troublesome dyskinesia such as proportion of ON-time without troublesome dyskinesia (Encarnacion and Hauser, 2008). These, unlike the traditional measures of the impact of dyskinesia employed in the majority of non-human primate studies, have been successfully employed in Phase III to provide a bridge through to the approval process and successful clinical use (Rascol *et al.*, 2005). Thus, a recent suggestion has been to incorporate measures of "good" ON-time, when there is reversal of parkinsonism with either no or non-disabling dyskinesia in contrast to "bad" ON-time when the animal exhibits a lack of parkinsonism but with disabling dyskinesia (Johnston *et al.*, 2010b, 2010c).

## E. Use of the Model

A multitude of studies have employed the MPTP-lesioned primate model to explore the pathogenesis underlying both parkinsonism and LID. These include investigations as to the effect of dopaminergic denervation on the electrophysiology of basal ganglia nuclei (Wichmann and DeLong, 2003) and pre- and post-synaptic molecular mechanisms (Calabresi *et al.*, 2010; Fasano *et al.*, 2010; Hallett *et al.*, 2005). In this regard the primate model excels in being the closest representation both anatomically and in expression of pathological behavioral phenotype, to the human disease state. However, post-mortem studies of this type present huge cost and logistical obstacles and most commonly are first investigated using rodent models. Perhaps more widely employed, the MPTP-lesioned primate remains without equal as an example of a neurological disease model with which to assess novel therapeutics with potential to improve parkinsonian disability, either as monotherapy or as an adjunct to L-DOPA. With such expectation placed upon it, the MPTP-primate's ability to inform and predict success in the clinic has been subjected to considerable scrutiny (reviewed by Fox *et al.*, 2006). The MPTP-primate has been employed to optimize use of L-DOPA or dopamine agonists and thus informed clinical dosing paradigms that minimize the development and/or expression or motor complications of treatment (Jackson *et al.*, 2007; Smith *et al.*, 2005). Use of non-dopaminergic therapies in PD long preceded that of L-DOPA lending credence to a vast array of subsequent studies demonstrating the role of glutamate, serotonin, noradrenaline, and other transmitters in the pathogenesis and expression of parkinsonian and dyskinetic behaviors. Table I serves to illustrate the considerable breadth of preclinical efficacy studies of novel therapeutics in the MPTP-primate and, where conducted, the equivalent clinical correlate. Examples of both translational successes such as the noradrenergic $\alpha_2$ antagonist, fipamezole (Dimitrova *et al.*, 2009; Johnston *et al.*, 2010c), and the adenosine $A_{2a}$ antagonists such as preladenant (Hauser *et al.*, 2010; Hodgson *et al.*, 2010) may be compared with application of opioid antagonists (Fox *et al.*, 2004; Henry *et al.*, 2001) or monoamine reuptake inhibitors Y (Frackiewicz *et al.*, 2002; Pearce *et al.*, 2002) that while still affording

Table I
Assessment of Select Approaches With Potential for the Treatment of L-DOPA-Induced Dyskinesia in Pre-clinical Studies and Man.

| Class and Receptor/ Target Subtype | Name of Compound | Demonstrated Ability to Evoke Reduced Dyskinesia or to Lessen L-DOPA-Induced Dyskinesia: | | | |
|---|---|---|---|---|---|
| | | 6-OHDA-lesioned rat | MPTP-lesioned primate | PD patient | Comment |
| Dopaminergic | | | | | |
| D1/D2/D3 agonist | Rotigotine | Y (Schmidt *et al.*, 2008) | Y (Stockwell *et al.*, 2009) | Y (Poewe *et al.*, 2007) | Not anti-dyskinetic per se but may evoke less dyskinesia than L-DOPA when given as monotherapy or in combination with L-DOPA |
| D2/D3 agonist | Pramipexole | Y (Larramendy *et al.*, 2008) | Y (Tayarani-Binazir *et al.*, 2010b) | Y (Fedorova and Chigir, 2007; Poewe *et al.*, 2007) | |
| | Cabergoline | Y (Larramendy *et al.*, 2008) | | | |
| | Ropinirole | Y (Carta *et al.*, 2008) | Y (Jackson *et al.*, 2007; Pearce *et al.*, 1998) | Y (Hauser *et al.*, 2007) | |
| D3 partial agonist | BP897 | Y (Visanji *et al.*, 2006) | Y (Bezard *et al.*, 2003; Hsu *et al.*, 2004) | | Not solely due to actions at D3R |
| D4 antagonist | L-745,870 | N/D | Y (Huot *et al.*, 2010a) | N/D | |
| DA reuptake/MAO-B inhibition | Safinamide | N/D | Y (Gregoire *et al.*, 2010) | Y (Meshram *et al.*, 2010; Schapira *et al.*, 2010) | |
| | Brasofensine | N/D | Y (Pearce *et al.*, 2002) | N (Frackiewicz *et al.*, 2002) | As for rotigotine |

| | | | | | |
|---|---|---|---|---|---|
| Mixed monoamine reuptake inhibitor | UWA-101 | N/D | Y (Huot *et al.*, 2010b) | N/D | |
| | SEP-228791 | N/D | Y (Johnston *et al.*, 2010a) | N/D | |
| COMT inhibitor | Entacapone | Y (Marin et al. 2006) | Y (Marin and Obeso, 2010; Smith *et al.*, 2005) | N (Hauser *et al.*, 2009) | May require more frequent administration to be effective |
| | Nebicapone | N/D | N/D | N (Ferreira *et al.*, 2010) | Increases ON-time only |
| MAO-B inhibitor | Rasagiline | N/D | N/D | Y (Parkinson-Study-Group, 2005) | Increases ON-time but needs careful L-DOPA titration to avoid concomitant increased LID Potential neuroprotective |
| Striatal dopamine receptor desensitization | GRK6 overexpression | Y (Ahmed *et al.*, 2010) | Y (Ahmed *et al.*, 2010) | N/D | |
| | Ras-GRF1 inhibition | (Fasano *et al.*, 2010) | Y (Fasano *et al.*, 2010) | N/D | |
| Noradrenergic $\alpha_{2a/2c}$ antagonists | Idazoxan | Y (Buck *et al.*, 2010) | Y (Domino *et al.*, 2003; Grondin *et al.*, 2000; Henry *et al.*, 1999) | Y (Rascol *et al.*, 2001) | |
| | Fipamezole | N | Y (Johnston *et al.*, 2010c; Savola *et al.*, 2003) | Y (Dimitrova *et al.*, 2009) | No ON-time extension in patients |
| | Rauwolscine | Y (Dekundy *et al.*, 2007) | Y (Henry *et al.*, 1999) | N/D | |
| | Clonidine | Y (Dekundy *et al.*, 2007) | Y (Gomez-Mancilla and Bedard, 1993) | N/D | |
| | Yohimbine | Y (Dekundy *et al.*, 2007) | Y (Gomez-Mancilla and Bedard, 1993) | N/D | |

(*Continued*)

Table I (*Continued*)

| Class and Receptor/ Target Subtype | Name of Compound | Demonstrated Ability to Evoke Reduced Dyskinesia or to Lessen L-DOPA-Induced Dyskinesia: | | | |
|---|---|---|---|---|---|
| | | 6-OHDA-lesioned rat | MPTP-lesioned primate | PD patient | Comment |
| Serotonergic | | | | | |
| 5-$HT_{1a}$ agonist (non-selective) | Sarizotan | Y (Gerlach *et al.*, 2011) | Y (Bibbiani *et al.*, 2001; Gregoire *et al.*, 2009) | Y (Bara-Jimenez *et al.*, 2005)<br>N (Goetz *et al.*, 2007) | May also extend ON-time<br>Also D3/4 antagonism activity |
| | Clozapine | N (Dekundy *et al.*, 2007) | Y (Grondin *et al.*, 1999b) | Y (Durif *et al.*, 2004) | |
| | 8-OHDPAT | Y (Carta *et al.*, 2007) | Y (Iravani *et al.*, 2006; Munoz *et al.*, 2008) | N/D | Worsens PD symptoms |
| | Pardoprunox | Y (Jones *et al.*, 2010) | Y (Tayarani-Binazir *et al.*, 2010a) | Y (Bronzova *et al.*, 2010) | |
| | Buspirone | Y (Dekundy *et al.*, 2007) | N/D | Y (Bonifati *et al.*, 1994) | |
| 5-$HT_{1b/1d}$ agonist | SKF-99101 | N/D | Y (Jackson *et al.*, 2004) | N/D | Worsens PD symptoms |
| 5-$HT_{2a}$ inverse agonist | Pimavanserin | N/D | Y (Vanover *et al.*, 2008) | N/D | Reduces psychosis in PD |
| 5-$HT_{2c}$ antagonist (mixed) | Quetiapine | N/D | Y (Oh *et al.*, 2002) | N (Katzenschlager *et al.*, 2004) | |
| | Methysergide | N/D | Y (Gomez-Mancilla and Bedard, 1993) | N/D | |
| 5-HT reuptake inhibition | Paroxetine | N/D | N/D | N (Chung *et al.*, 2005) | |
| | Fluoxetine | N (Dekundy *et al.*, 2007) | N/D | N/D | |
| | Fluvoxamine | N/D | N (Iravani *et al.*, 2003) | N/D | |

| | | | | | |
|---|---|---|---|---|---|
| Glutamatergic | | | | | |
| Non-selective NMDA antagonist | Amantadine | Y (Dekundy *et al.*, 2007; Lundblad *et al.*, 2002) | Y (Blanchet *et al.*, 1998; Hill *et al.*, 2004) | Y (Sawada *et al.*, 2010; Snow *et al.*, 2000; Verhagen Metman *et al.*, 1998b) | |
| | LY235959 | N/D | Y (Papa and Chase, 1996) | N/D | |
| NR2a-selective NMDA antagonist | MDL 100,453 | N/D | N (Blanchet *et al.*, 1999) | N/D | |
| NR2b-selective NMDA antagonist | Traxoprodil | N/D | N (Nash *et al.*, 2004) | Y (Nutt *et al.*, 2008) | Risk of cognitive deficits |
| | Besonprodil | N/D | Y (Morissette *et al.*, 2006) | N/D | |
| AMPA antagonist | Talampanel | N/D | Y (Bibbiani *et al.*, 2005) | N/D | |
| | LY300164 | N/D | Y (Konitsiotis *et al.*, 2000) | N/D | (-) enantiomer of talampanel |
| | IEM-1460 | Y (Kobylecki *et al.*, 2010) | Y (Kobylecki *et al.*, 2010) | N/D | GluR2-lacking, $Ca^{2+}$-permeable selective, some NMDA blockade |
| mGlu5 negative allosteric modulator | MTEP | Y (Mela *et al.*, 2007; Rylander *et al.*, 2009) | Y (Johnston *et al.*, 2010b; Morin *et al.*, 2010) | N/D | May worsen PD symptoms |
| | MPEP | Y (Jimenez *et al.*, 2009; Yamamoto and Soghomonian, 2009) | Y (Morin *et al.*, 2010) | N/D | |
| | Fenobam | Y (Rylander *et al.*, 2010a) | Y (Rylander *et al.*, 2010a) | N/D | |
| | ADX 48621 | N/D | Y (Hill *et al.*, 2010) | N/D | Phase II ongoing |
| | AFQ056 | N/D | Y (Gregoire *et al.*, 2008) | Y (Berg *et al.*, 2010) | Phase II ongoing |
| Exogenous cannabinoids | | | | | |
| CB1 agonist | Nabilone | N/D | Y (Fox *et al.*, 2002) | Y (Sieradzan *et al.*, 2001) | |
| | WIN 55,212-2 | Y (Morgese *et al.*, 2007) | N/D | N/D | |

(*Continued*)

Table I (*Continued*)

| Class and Receptor/ Target Subtype | Name of Compound | Demonstrated Ability to Evoke Reduced Dyskinesia or to Lessen L-DOPA-Induced Dyskinesia: | | | |
|---|---|---|---|---|---|
| | | 6-OHDA-lesioned rat | MPTP-lesioned primate | PD patient | Comment |
| CB1 inverse agonist | Rimonabant | N (Walsh *et al.*, 2010) | Y (van der Stelt *et al.*, 2005) | N (Mesnage *et al.*, 2004) | |
| Opioids | | | | | |
| Non-specific opioid antagonist | Naloxone | Y (Dekundy *et al.*, 2007; Lundblad *et al.*, 2002) | Y (Klintenberg *et al.*, 2002)<br>N (Samadi *et al.*, 2003) | N (Fox *et al.*, 2004) | |
| μ-opioid antagonist | Naltrexone | N/D | Y (Henry *et al.*, 2001)<br>N (Samadi *et al.*, 2003; Tamim *et al.*, 2010) | N (Manson *et al.*, 2001; Rascol *et al.*, 1994) | |
| | Cyprodime | N/D | Y (Henry *et al.*, 2001) | N/D | |
| | ADL5510 | N/D | Y (Fox *et al.*, 2010) | N/D | |
| δ-opioid antagonist | Naltrindole | N/D | Y (Henry *et al.*, 2001) | N/D | |
| | Nor-binaltorphimine | N/D | Y (Henry *et al.*, 2001) | N/D | |
| κ-opioid agonist | U50, 488 | N/D | Y (Cox *et al.*, 2007) | N/D | May worsen PD symptoms |
| | TRK-820 | Y (Ikeda *et al.*, 2009) | N/D | N/D | May worsen PD symptoms |
| Adenosinergic | | | | | |
| $A_{2a}$ antagonist | Istradefylline | Y (Lundblad *et al.*, 2003; Spinnewyn *et al.*, 2010) | Y (Bibbiani *et al.*, 2003; Grondin *et al.*, 1999a) | Y (Bara-Jimenez *et al.*, 2003; Kanda *et al.*, 2000) | |
| | Preladenant | N/D | Y (Hodgson *et al.*, 2010) | Y (Hauser *et al.*, 2010) | |
| | Vipadenant | N/D | N/D | Y (Papapetropoulos *et al.*, 2010) | Efficacious but toxicity issues |
| $A_{2a}/A_1$ antagonist | ASP5854 | N/D | Y (Mihara *et al.*, 2008) | N/D | |

| | | | | | |
|---|---|---|---|---|---|
| Histaminergic | | | | | |
| $H_2$ antagonist | Famotidine | N/D | Y (Johnston *et al.*, 2010e) | Y (Molinari *et al.*, 1995) | Reduces chorea exacerbates dystonia |
| $H_3$ agonist | Immepip/Imetit | N/D | Y (Gomez-Ramirez *et al.*, 2006) | N/D | Reduces chorea only |
| Cholinergic | | | | | |
| Nicotinic agonist | NP002 (nicotine dehydrate bitatrate) | N/D | Y (Quik *et al.*, 2007) | Y (Neuraltus, 2010) | Phase II ongoing |
| | Varenicline | N (Huang *et al.*, 2011) | N/D | N/D | |
| | Mecamylamine | Y (Bordia *et al.*, 2010)<br>N (Dekundy *et al.*, 2007) | N/D | N/D | Effects only found with chronic treatment, not acute |
| | A-85380 | Y (Huang *et al.*, 2011) | N/D | N/D | Phase II ongoing |
| Miscellaneous | | | | | |
| SV2a | Levetiracetam | N/D | Y (Bezard *et al.*, 2004) | Y (Stathis *et al.*, 2010) | |

**Key**: Y: Reduced expression of dyskinesia (either as monotherapy or in combination with L-DOPA) N: No effect of drug on dyskinesia N/D: Not determined

considerable preclinical promise have yet to be successfully applied in the clinic. Exhaustive commentary specifically regarding development of novel therapeutics for dyskinesia in PD may be found in accompanying chapters of this book and elsewhere (Buck and Ferger, 2010; Fox and Brotchie, 2010; Fox *et al.*, 2006).

## III. Unilateral 6-OHDA-Lesioned Rodent Model of L-DOPA-Induced Dyskinesia

### A. Introduction

The considerable strengths of the primate models of LID support their primary use in the translation of new anti-parkinsonian or anti-dyskinetic agents to the clinic. However, in contrast to the rodent, their widespread use in understanding mechanisms of LID is often limited by logistical and ethical concerns (indeed the use of primates in Europe is becoming significantly more limited under new legislation). Rodents can be used in greater abundance to create highly controlled and homogenous experimental groups. Nevertheless the rodent model has only been a relatively recent addition to the dyskinesia field, and has fought considerable controversy centered largely on the accuracy and relevance of the evoked behaviors. It has now become widely accepted as an additional tool in the need to understand PD and LID, albeit with limitations, many of which also apply to the MPTP-lesioned primate.

From its inception in the late 1960s the 6-OHDA lesioned rat model has been widely used in the study of PD. This toxin, administered stereotaxically as it cannot cross the blood–brain barrier, is taken up through the catcholaminergic transporter causing degeneration of catecholaminergic neurons thought to be through increased oxidative stress. Complete bilateral dopaminergic depletion, as caused by MPTP in primates, can be obtained by the administration of 6-OHDA into the lateral ventricles but this has a significant impact on the health and wellbeing of the animal, inducing adipsia and aphagia (Ungerstedt, 1971a) and is therefore rarely used today. These consequences, however, can be avoided by the unilateral stereotaxic injection of 6-OHDA directly into the nigrostriatal tract. Direct administration into the substantia nigra produces rapid cell death within days whilst if targeted to the medial forebrain bundle (containing the nigrostriatal dopaminergic projections) retrograde cell death takes a little longer, and longer still if the toxin in injected into the terminal regions in the striatum (about 2–3 weeks). The result is a dopaminergic lesion on one side of the brain, leaving the animal capable of maintaining itself fully but with lateralized motor and sensory impairments (Ungerstedt and Arbuthnott, 1970). Typically it requires a specific behavioral task or challenge with a dopaminomimetic agent to reveal these lesion-induced deficits in motor behavior and batteries of such

tests are well described elsewhere (reviewed by Schwarting and Huston, 1996). The extent and topographical location of the lesion determine the specific nature and degree of the deficits, as well as the level of improvement attained by therapeutic intervention. However, the primary use of this model has been in the exploitation of the rotational locomotor asymmetry caused by the dopamine imbalance, an easily measured indicator of dopaminergic activity. The indirect acting dopamine agonist, amphetamine, increases synaptic dopamine in the intact hemisphere, but is unable to do so in the dopamine depleted striatum, evoking robust ipsiversive rotations that are correlated with lesion extent (Ungerstedt, 1971c). In contrast, direct acting dopamine agonists and L-DOPA preferentially stimulate dopamine receptors that have become supersensitive in the lesioned hemisphere, thus evoking contraversive rotations (Ungerstedt, 1971b). This continues to be used as a screening tool for the anti-parkinsonian potential of new agents but formerly had a limited relationship with assessment of dyskinesia. The dyskinesiogenic potential of new ligands has been evaluated against parameters of the rotational behavior observed in the chronically L-DOPA treated rat, that is the response to L-DOPA becomes more rapid in onset and increases in magnitude and some groups have found a reduced response duration compared to the first day of treatment (Bevan, 1983; Deshaies *et al.*, 1984). This change in responsiveness (rapid onset and increased rate) is reminiscent of the behavioral sensitization observed in non-human primates and PD patients, while the reduced duration has been likened to the "wearing-OFF" effect. "Wearing-OFF" is another complication of chronic L-DOPA administration where patients require medicating at more frequent intervals as the efficacy of L-DOPA wanes (Stocchi *et al.*, 2008). It is now clear that these characteristics are not good parameters against which to judge dyskinetic potential, as dopamine $D_2$ receptor preferring agonists have limited potential to induce dyskinesia in the clinic, but will cause pronounced changes in rotational behavior in rats over time. In 1998, the first paper was published by Cenci and colleagues, who demonstrated that beyond circling behavior rats also display abnormal involuntary AIMs in response to long-term administration of L-DOPA (Cenci *et al.*, 1998). The behaviors were described as torsional twisting of the torso, dystonic, and hyperkinetic movements of the forelimb and rapid chewing motion of the orolingual area with tongue protrusions. Using rating scales in a similar fashion to the MPTP-lesioned primate, these behaviors can be scored based on their presence and severity over the course of activity of L-DOPA or other pharmacological agent.

### B. Generation of L-DOPA-Induced Dyskinesia in the Rodent

AIMs in the rodent are generated through the subchronic administration of L-DOPA in combination with an aromatic acid decarboxylase inhibitor. The ideology is clearly to be clinically relevant therefore low starting doses of

L-DOPA are generally preferred (Cenci and Lundblad, 2007) although some groups advocate high doses in the early stages of treatment then reducing after AIMs have been established (Steece-Collier *et al.*, 2003). Initial treatments typically should evoke a mild contraversive rotational response and be sufficient to restore motor function to the impaired side of the body as determined through symmetrical paw use in the cylinder test or performance on the rotarod (Lundblad *et al.*, 2002). Movements become increasingly pronounced and are present for a greater proportion of the time "ON" L-DOPA. A stable response to L-DOPA at a single dose is commonly obtained after 2–3 weeks of drug administration by which point the dose can be increased if AIMs are not sufficiently severe. Once a stable state has been reached this responding remains remarkably consistent, in both overall severity and the distribution of AIMs across the subtypes measured (e.g., orolingual or limb) and administration can be dropped to once or twice a week. The variability of absorption and metabolism of oral administration is avoided by the use of the methyl ester of L-DOPA, most commonly administered through a parenteral route (typically subcutaneous). Initial studies used interperitoneal administration and some suffered from a high degree of variability when some animals failed to respond during particular trials. This "dose failure" rate was abolished by the now preferred use of subcutaneous L-DOPA administration, giving similar behavioral motor responses but with a slight shift in time course (Lindgren *et al.*, 2007).

The location along the nigrostriatal pathway at which the lesion is applied is not of primary importance but, as with patients and the primate model, the development of dyskinesia is in part determined by the extent of the dopamine depletion (Winkler *et al.*, 2002). Striatal lesions, allowing the preservation of limbic accumbal dopaminergic innervation can require several toxin deposits to produce the 80% or more dopamine depletion necessary for the generation of AIMs although the locomotor component will be less pronounced using this approach. These methods have also been applied in 6-OHDA lesioned mice and extensively validated in both species. One important consideration in testing this model is that the rotational response to amphetamine and apomorphine is a standard approach to the assessment of lesion extent. Apomorphine, a non-selective $D_1/D_2$ dopamine agonist is capable itself of producing significant sensitization and AIMs after single doses and therefore cannot be used to test evaluate lesion extent in AIMs experiments. "Non-dyskinetic" animals are defined as having rotational responses and therefore being motorically active in response to L-DOPA but with minimal or no abnormal movements. Approximately 20% of any cohort will be classified as non-dyskinetic (showing few or no dyskinetic movements in response to L-DOPA but with a sustained locomotor response) and this has not been related to the extent of dopaminergic depletion or other conclusively identifiable parameter thus far. As a result of the large group numbers that can been employed in rat experiments (compared to primates) this inherent variability can be exploited; non-dyskinetic animals may either be removed from the study or used as a control cohort.

### C. Rating Scales

In a similar approach to human and primate dyskinesia assessments the basis for the study is a subjective rating scale. The first reported rating scale for dyskinesia assessment in rodents was based on the duration of expression of axial, orolingual, and forelimb behaviors, scoring 0 for absent, 1 if it was present for less than half time, 2 if present for more than half the time, 3 if present for the whole time but interruptible by an external stimulus (e.g., a sharp tap on the cage), and 4 if uninterruptible by said stimulus. This scoring is repeated at regular intervals throughout the duration of L-DOPA activity (2–4 h depending on dose). Locomotion is also scored, and specifies deliberate locomotion in a circular pattern, as opposed to the "rotation" counts generated by an automated rotometer that may include movements generated by extreme axial twisting and loss of balance. The measure of locomotor AIMs is therefore able to provide an alternate index of rotational behavior and is particularly useful if testing is not performed in automated rotometers. However, this temporal based scoring criterion fails to recognize the development of the movements themselves, the quality of which changes over the course of chronic L-DOPA treatment. For example, forelimb movements, especially following the first few doses of low dose L-DOPA, are typically small oscillations of the paw and distal forelimb around a fixed position, but may develop into more vigorous limb and shoulder movements, occasionally ballistic in character as treatment progresses. Winkler and colleagues added a layer of complexity and greater dynamic range of the scale by the inclusion of "amplitude" scores, which take into account these movement changes in forelimb, axial, and orolingual dimensions (Winkler *et al.*, 2002). An additional component that can also be added to this score is that of hindlimb dystonia, assessing the extension, rotation and elevation of the hindlimb. An overall score reflecting dyskinesia severity can then be obtained from a combination of the different parameters (Cenci and Lundblad, 2007). This scale is now remarkably similar to the clinical AIMs rating scale described by Guy (1976), one of only two clinical scales recommended for use in clinical trial assessment of dyskinesia severity in patients (Colosimo *et al.*, 2010; Guy, 1976).

In a similar approach, a slightly different scoring method was proposed in 2003 by Steece-Collier and co-workers that also incorporates measures of duration and amplitude generally applied at one or two time-points post-administration of L-DOPA. This scale includes a greater number of parameters and also attempts to classify components of the behaviors as dystonic or hyperkinetic, two distinct elements of the clinical syndrome of dyskinesia (Maries *et al.*, 2003; Steece-Collier *et al.*, 2003). This is clearly advantageous but with the greater number of parameters it is technically challenging to score live and is not commonly used. Whether these elements can truly be distinguished in a rodent model in combination with locomotion and other complex movements is questionable and other

elements in this scale have not been as rigorously validated against pharmacological probes as has been applied by Cenci and co-workers.

### D. Use of the Model

Rotational behavior remains a popular measure of dopaminergic or anti-parkinsonian activity and in some cases dyskinesiogenic potential (Henry *et al.*, 2003; Visanji *et al.*, 2009a) but the arguments against rotation as measure of dyskinesia actually work to support the AIMs model. As described above, the chronic administration of selective $D_2/D_3$ dopamine receptor agonists will induce the sensitization of rotational behavior in a similar fashion to L-DOPA (Lane and Dunnett, 2010; Lindgren *et al.*, 2007; Ravenscroft *et al.*, 2004), yet clinically, dopamine agonists are associated with significantly lower levels of dyskinesia when administered *de novo* (Perez-Lloret and Rascol, 2010). However, the quality of the circling behavior differs, circling being more "on the spot" small diameter turns with a twisted torso in the center of the bowl following apomorphine or L-DOPA. On the other hand, dopamine agonists such as bromocriptine produce contralateral amphetamine-like locomotion, circling with large diameter, and a straighter torso around the perimeter of the bowl (Koshikawa, 1994). Thus the current interpretation of the behavior of the rodent model is that rotation is a useful index of functional recovery, whilst the development of abnormal movements is representative of dyskinesia. The litmus test in the validation of this model as a screen for potential anti-dyskinetic agents is that when co-administered with L-DOPA there is a reduction in the severity of abnormal movements but importantly, with the preservation of the circling behaviors indicating that L-DOPA retains its anti-parkinsonian efficacy. A major criticism of the dyskinesia model has been that the behaviors are simply unilateral stereotypies. However, whilst amphetamine induces significant stereotypy, LID-like movements are not observed, suggesting that this is specific to the changes that occur in the denervated striatum. We have found that if behaviors are assessed using the Creese and Iversen stereotypy scale (Creese and Iversen, 1973) there is no significant change over chronic L-DOPA usage, in contrast to AIMs that increase in severity. The Cenci scale of abnormal movements has been extensively validated in both 6-OHDA lesioned rats and mice using known anti-dyskinetic agents such as amantadine and to evaluate putative agents such as 5-$HT_{1A}$ agonists, metabotropic glutamate receptor antagonists, opioid agonists, and antagonists (for a more comprehensive list see Table I) (Dekundy *et al.*, 2007; Lundblad *et al.*, 2002, 2004, 2005). Many of these have been either previously or subsequently identified as alleviating LID in the MPTP-lesioned primate, although translation into clinical trials has been more variable (See Table I and reviewed extensively in Fox *et al.*, 2006). As with the MPTP-lesioned primate the model can be used either to assess anti-parkinsonian efficacy,

use as an adjunct to L-DOPA, or evaluated purely as an anti-dyskinetic agent. Most agents are evaluated in all three paradigms, given *de novo*, co-administered with L-DOPA over several weeks or given in single pulses to rats with established dyskinesia generated through several weeks of L-DOPA treatment until stabilized.

Neurochemical evaluations have often taken advantage of the unilateral nature of the model, using the intact hemisphere as a control, this should be done with caution as recent studies highlight the bilateral changes that can occur as a result of the lesion (Pierucci *et al.*, 2009) and intact or lesion only control groups would always be valuable. Many of the identified changes in striatal neurochemistry following chronic L-DOPA administration are comparable to the MPTP-lesioned primate, some of which have also been examined in PET scans or post-mortem analysis of dyskinetic patients, supporting the use of the rodent model in studies of underlying mechanistic changes. Key examples of this include the elevated striatal expression of pre-proenkaphlin B and $\delta$-FosB-like proteins in rodents and primates with LID and in patients with PD that have been treated with L-DOPA (Andersson *et al.*, 1999; Doucet *et al.*, 1996; Henry *et al.*, 2003; Tekumalla *et al.*, 2001). In distinguishing the beneficial effects of L-DOPA from LID, the presence of non-dyskinetic animals has been hugely facilitatory in the continued understanding of dyskinesia. The rodent model has enabled full proteomic and mRNA microarray screens of dyskinetic, non-dyskinetic, and comparable dopamine agonist-treated groups (Konradi *et al.*, 2004; Valastro *et al.*, 2007). The full potential of this model has yet to realized with the increasing availability of transgenic and knock-out technologies in the search for the causative mechanisms of LID but increasingly collaborative studies are emerging in which mechanistic clues are obtained in the rodent models and then evaluated in the primate.

## IV. Critique of Toxin-Based Models of L-DOPA-Induced Dyskinesia

As much as the behavioral phenotype induced by L-DOPA treatment in the MPTP-lesioned primate clearly resembles dyskinesia in PD there are limits to its ability to predict therapeutic efficacy in the clinic and to model all pathological processes underlying both LID and PD. Most obviously, cell death and development of parkinsonian symptoms in PD are ongoing processes and 6-OHDA and MPTP paradigms, whilst able to cause slow (MPTP) or partial cell loss (both) are not truly "progressive". Patterns of aberrant protein aggregation and neuronal loss particularly in regard to deposition of $\alpha$-synuclein also differ between human, primate, and rodent (Braak *et al.*, 2003). In that capacity, the 6-OHDA lesioned rat does not show significant accumulation of $\alpha$-synuclein, while in the primate $\alpha$-synuclein pathology can occur, but to a lesser extent that that seen

in PD patients. In the parkinsonian baboon, increased intracellular α-synuclein has been shown within the substantia nigra although it is not complexed into Lewy-like aggregates (Kowall *et al.*, 2000). The perceived absence of Lewy bodies in the MPTP-lesioned primate is unlikely due to inspection of the tissue being made too close to the time of MPTP-administration since a lack of Lewy pathology has also been witnessed over a decade following MPTP (Halliday *et al.*, 2009). Instead, it is possible that the relatively rapid kinetics of MPTP-induced cell-death combined with differences in the array of causes of idiopathic PD compared to those exerted by MPTP underlie the lack of Lewy pathology.

Post-mortem and imaging studies of PD patients have demonstrated that degeneration of the nigrostriatal dopaminergic system, whilst being the dominant pathological feature of the disease, is not the sole degenerative process occuring in this disorder. Noradrenaline, serotonin, and acetylcholine are all affected in PD albeit to a lesser degree than dopamine (Fahn *et al.*, 1971; Javoy-Agid *et al.*, 1984; Jellinger, 1991). Levels and patterns of brain dopamine loss might bear close comparison between PD and the MPTP primate particularly in striatal regions but those of key non-dopaminergic transmitters, noradrenaline and 5-HT show considerable variations between the two (Pifl *et al.*, 1991). In the case of the rodent model, the surgical process involved in the lesion into the medial forebrain bundle can damage noradrenergic and serotonergic projections to varying degrees. Targeting the dopaminergic cell bodies in the substantia nigra or the terminal areas in the striatum can avoid the loss of noradrenergic forebrain innervation but other projection areas may still be affected. Typically the nigral and striatal lesions produce a less complete lesion but adequate dopaminergic depletion can be achieved with multiple injection sites. To minimize non-dopaminergic cell loss, noradrenaline and serotonin reuptake blockers are administered prior to surgery to prevent uptake of the toxin and slower infusion speeds have been used to limit non-specific reuptake or damage to other projection fibres in the vicinity of the injection site. The ability to selectively lesion not only dopaminergic systems but also noradrenaline and serotonin (with the use of DSP-4 and 5'7-DHT respectively) has been taken advantage of in the 6-OHDA lesioned rodent and to specifically explore their contribution to the development of LID (Carta *et al.*, 2007; Perez *et al.*, 2009). Selective destruction of the serotonergic system has revealed that in the absence of these terminals in the striatum, LID does not develop but at the same time L-DOPA no longer produced a functional effect (Tanaka *et al.*, 1999). L-DOPA requires conversion into dopamine by amino acid decarboxylase and it is now becoming widely accepted that this may occur in serotonergic terminals in the striatum (Carta *et al.*, 2007; Rylander *et al.*, 2010b). In addition, prolonged dopamine replacement therapy in 6-OHDA-lesioned animals has recently been shown to evoke increased sprouting of striatal serotonergic terminals (Rylander *et al.*, 2010b). Dopamine is then stored in serotonergic vesicles and its release is no longer under the control of dopaminergic feedback systems and

this dysregulation contributes to the receptor sensitization that leads to dyskinesia (see commentary by Picconi *et al.*, 2010).

The continued ability of the animals to sustain themselves is the primary reason for the popularity of the unilateral 6-OHDA lesioned rat compared to bilateral versions (Ungerstedt, 1971a). However, the laterality of the behavior and rotational tendency can complicate the measurement of functional recovery. If the animal is severely dyskinetic in the presence of L-DOPA or other pharmacological agent, their performance in simple and more complex behavioral tasks can be hindered, for example identifying deliberate forelimb use in the cylinder test when an animal is rotating and moving one limb excessively is difficult and can lead to false positive scores.

Another caveat to the rodent model is the ease with which LID can become associated with the environment (Lane *et al.*, 2011). The sensitization of the rotation and development of dyskinesia is certainly in part due to the pharmacological changes occurring through the basal ganglia, but an element of the observed change in responsivity may be due to a process termed context-specific sensitization. Groups of rats that were exposed to a particular environment during chronic L-DOPA administration developed dyskinesia as expected, but when they were placed in a familiar but different environment, the severity of dyskinesia dropped significantly. In particular the rapid onset of the response is not sustained and the peak severity of the response is reduced. Given that many drugs are not currently translating into the clinic this could be one of the mechanisms through which artifacts are being introduced into studies and it remains fundamental to the study of LIDs that animals are exposure to testing environments frequently in the absence of drug administration to limit the impact of this association.

## V. Alternative Models of L-DOPA-Induced Dyskinesia

The therapeutic predictive validity of the 6-OHDA lesioned rodent and particularly the MPTP-lesioned primate models, when applied appropriately, remains impressive though not without room for improvement. Alternative animal models for the study of LID that better mimic underlying disease pathogenesis have been sought. However, there remain few in number, largely because they fail to show significant levels of dopamine depletion. Since it has been established that in PD the extent of dopamine depletion is largely associated with the risk of developing LID, and that rodent models do not develop LID in the absence of dopamine depletion this aspect of the model is fundamental. There is one current exception to this and that is the transgenic line of mice lacking the gene encoding the transcription factor, pituitary homeobox-3

(PITX3) (Nunes *et al.*, 2003). This is one of the key genes in the development of dopaminergic neurons and these mice lack A9 nigral and to some extent their ventral tegmental (A10) neurons. PITX3 deficient mice have motor deficits that are reminiscent of PD that can be ameliorated by dopamine replacement while the sub-chronic administration of L-DOPA reportedly is able to induce LID (Ardayfio *et al.*, 2010; Ding *et al.*, 2007). They do, however, show some paradoxical behaviors in response to anti-psychotic stimulation, which may indicate that other dopaminergic systems are altered (Ardayfio *et al.*, 2010). Importantly they also show changes in biochemical markers that have been associated with LID development such as increased ΔfosB and pre-prodynorphin expression in the striatum (Ding *et al.*, 2007).

## VI. Future Modeling of L-DOPA-Induced Dyskinesia

Current animal models of dyskinesia suffer from the same criticisms that are levied at the underlying representation of parkinsonism on which they are based. Thus, their face validity is reasonable but the construct validity is flawed. The pathophysiology of PD is far more complex and diffuse than the nigrostriatal dopamine loss (Braak *et al.*, 2003) and the presence of Lewy bodies that are commonly used as the definitive post-mortem diagnosis. As discussed above, the role of other neural pathways in the pathogenesis of the disease is not fully understood nor well represented in current models of the disease. Similarly, neither model reproduces the protein inclusion pathology. The role of α-synuclein in the development of dyskinesia remains undetermined but there is evidence that α-synuclein could be involved in the regulation of synaptic neurotransmitter release (Nemani *et al.*, 2010).

Increasingly the understanding of the genetics of PD is starting to lead to new animal models, providing what are hoped to be more disease-specific progressive modeling of the pathophysiology of PD. However, a significant problem with the transgenic models produced thus far is that they do not develop significant dopaminergic depletion and motor deficits that do develop have instead been attributed to degeneration at the level of the spinal cord (Mendritzki *et al.*, 2010). The use of viral vector-driven administration of wild-type or mutant α-synuclein (Kirik *et al.*, 2002; Koprich *et al.*, 2010; Ulusoy *et al.*, 2010) or common mutations of LRRK2 to specifically target dopaminergic neurons ARE being used (Dusonchet *et al.*, 2011). Nevertheless, the significant roadblock to the use of these models in dyskinesia research is the lack of dopaminergic denervation. A 70–80% loss of striatal dopamine is considered necessary before dyskinesia will be evoked and these models do not consistently give that level of deficit.

## VII. Conclusions

Progress in the understanding and treatment of complications of dopamine replacement therapy in PD owes much to the discovery and development of animal models of the condition. Unequivocally, the MPTP-lesioned primate remains the definitive model of parkinsonian motor symptoms as well as L-DOPA-induced dyskinesia in PD. Its continued use in translating pre-clinical findings into therapeutically useful treatments will be enhanced as experimental designs increasingly take stock of clinically relevant outcome measures. Meanwhile, the 6-OHDA-lesioned rat and other models currently in development will continue to be pivotal in adding to our understanding of the pathophysiological mechanisms responsible for dyskinesia in PD.

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# MOLECULAR MECHANISMS OF L-DOPA-INDUCED DYSKINESIA

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Parkinson's disease (PD), a common neurodegenerative disorder caused by the loss of the dopaminergic input to the basal ganglia, is commonly treated with L-DOPA. Use of this drug, however, is severely limited by the development of dystonic and choreic motor complications, or dyskinesia. This chapter describes the molecular mechanisms implicated in the emergence and manifestation of L-DOPA-induced dyskinesia (LID). Particular emphasis is given to the role played in this condition by abnormalities in signal transduction at the level of the medium spiny neurons (MSNs) of the striatum, which are the principal target of L-DOPA. Recent evidence pointing to pre-synaptic dysregulation is also discussed.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00004-3





0074-7742/10 $35.00

## I. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by the death of midbrain dopaminergic neurons located in the substantia nigra pars compacta (SNc) and projecting to the dorsal striatum (Hornykiewicz, 1963). The loss of striatal dopamine results in the appearance of the cardinal symptoms of PD, which include tremor, rigidity, and hypokinesia. These symptoms are commonly treated with the dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) and dopamine agonists, which, in the early phase of the disease, effectively reverse motor impairment (Birkmayer and Hornykiewicz, 1998; Cotzias *et al.*, 1967; Mercuri and Bernardi, 2005). However, prolonged use of many of these drugs, in parallel to the progressive degeneration of nigrostriatal neurons, results in the appearance of motor complications including dystonic and choreic movements generally referred to as dyskinesia (Fabbrini *et al.*, 2007; Obeso *et al.*, 2000).

The development of dyskinesia is particularly problematic in combination with administration of L-DOPA, which still represents the most effective pharmacological approach to the treatment of PD (Mercuri and Bernardi, 2005). Several factors have been proposed to contribute to the development of L-DOPA-induced dyskinesia (LID) including age at PD onset (Kumar *et al.*, 2005), duration of disease and higher cumulative doses of L-DOPA (Hauser *et al.*, 2006; Schrag and Quinn, 2000). In addition, pre-clinical studies show that treatment regimens that avoid pulsatile stimulation of dopamine receptors reduce the risk for LID (Di Monte *et al.*, 2000; Jenner, 2004).

Clinical studies indicate that resolving LID in patients with advanced stages of PD would represent a significant improvement of their quality of life (Damiano *et al.*, 2000; Pechevis *et al.*, 2005). Moreover, the risk of developing drug-induced dyskinesia often leads clinicians to prescribe doses of medications insufficient to provide a full anti-parkinsonian effect. For these reasons, the possibility to combine dopaminergic drugs with substances able to prevent the development of dyskinesia would represent a considerable progress in the current pharmacotherapy of PD.

At present, amantadine, an antagonist at the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, is the drug most often used for the treatment of LID (Goetz *et al.*, 2005). Dyskinesia is also treated with deep brain stimulation of the globus pallidus and the subthalamic nucleus (Metman and O'Leary, 2005, The Deep-Brain Stimulation for Parkinson's Disease Study Group, 2001). These approaches are challenged by side effects that include confusion and exacerbation of hallucinations, and by potential complications related to the surgical procedure necessary to implant deep brain stimu-lators. An improvement in the interventions aimed at reducing the emergence or the expression of dyskinesia in parkinsonian patients is therefore highly desirable.

A first critical issue in the study of the mechanisms at the basis of LID is the identification of the plastic rearrangements produced in the brain by the loss of nigrostriatal neurons, particularly during advanced stages of PD. These changes alter dramatically the basic properties of specific groups of neurons and, most importantly, modify their ability to respond to dopaminergic drugs, including L-DOPA. A second important point is to establish which of these changes contribute to the development and to the maintenance and expression of dyskinesia. During the last years, considerable progress has been made along these directions, primarily through the combined use of non-human primate and rodent models of PD and LID (Bézard *et al.,* 2001; Cenci *et al.,* 2002; Jenner, 2008).

## II. Basal Ganglia and Medium Spiny Neurons

The study of the mechanisms of LID is strictly related to the characterization of the effects exerted by L-DOPA and other anti-parkinsonian drugs at the level of the basal ganglia, a collection of subcortical structures involved in the control of motor function. The striatal formation, which includes the dorsal (caudate-putamen) and the ventral striatum (nucleus accumbens), is the major component of the basal ganglia. The dorsal striatum is densely innervated by dopaminergic fibers originating in the SNc, which modulate glutamatergic excitatory inputs from cortical, thalamic, and limbic areas. This signal integration occurs for the most part on the dendritic arborization of GABAergic medium spiny neurons (MSNs), the principal neuronal type in the striatum (Albin *et al.,* 1989; Alexander *et al.,* 1986; Gerfen, 1992).

In the dorsal striatum, MSNs can be distinguished on the basis of their connectivity to the output stations of the basal ganglia (globus pallidus pars interna and substantia nigra pars reticulata). One group of MSNs innervates directly these structures, whereas the other projects to these nuclei indirectly, via globus pallidus pars externa and subthalamic nucleus. Because of this difference in connectivity, it is generally assumed that activation of the neurons of the "direct," or striatonigral, pathway promotes motor activity via disinhibition of thalamo-cortical neurons, whereas activation of the neurons of the "indirect," or striatopallidal, pathway suppresses motor activity by increasing inhibition on thalamo-cortical neurons (Albin *et al.,* 1989; Alexander *et al.,* 1986; Gerfen, 1992).

Dopamine promotes motor activity by exerting an excitatory effect on the MSNs of the direct pathway and, concomitantly, by reducing the activity of the MSNs of the indirect pathway. These regulations are brought about by acting on two G-protein-coupled receptors (GPCRs): the dopamine D1 receptor (D1R), which is selectively expressed in the direct pathway, and the dopamine D2 receptor

(D2R), which is expressed in the indirect pathway (Gerfen, 1992). The contrasting actions of D1Rs and D2Rs on the activity of MSNs are in line with the coupling of these receptors to different G-proteins, which exert opposite regulations on adenylyl cyclase (AC), the enzyme responsible for the conversion of ATP into cAMP. Activation of D1Rs leads to Gαolf-mediated stimulation of AC and increased cAMP, whereas activation of D2Rs leads to Gαi/o-mediated inhibition of AC (Herve *et al.*, 1993; Stoof and Kebabian, 1981; Zhuang *et al.*, 2000). The distinct functional roles played by the MSNs of the direct and indirect pathways in motor function, as well as the different control exerted by dopamine on these neurons, indicate the importance of identifying changes occurring specifically in one or the other neuronal population.

### III. LID and Hyperactivity of D1R/cAMP Signaling

In PD, the loss of dopamine caused by the degeneration of SNc neurons results in a dramatic increase in the responsiveness of striatal MSNs to dopaminergic drugs, including L-DOPA. For instance, the ability of dopamine to stimulate AC via D1Rs is enhanced in parkinsonian patients and in experimental animals lesioned with 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), two toxins commonly employed to generate experimental models of PD (Corvol *et al.*, 2004; Mishra *et al.*, 1974; Pifl *et al.*, 1992a, 1992b). The hyper-responsiveness produced by the loss of dopamine, which most likely represents a compensatory mechanism to counteract the lack of striatal dopamine, cannot be accounted for by changes occurring at the receptor level. In fact, the number and affinity of striatal D1Rs is not increased in rodents lesioned with 6-OHDA, in monkeys intoxicated with MPTP (Aubert *et al.*, 2005; Breese *et al.*, 1987; Joyce, 1991; Marshall *et al.*, 1989; Savasta *et al.*, 1988), or in parkinsonian patients (Hurley *et al.*, 2001; Pimoule *et al.*, 1985; Shinotoh *et al.*, 1993).

Studies performed in 6-OHDA lesioned rats and in post-mortem samples from parkinsonian patients demonstrated that loss of striatal dopamine is accompanied by increased levels of Gαolf (Corvol *et al.*, 2004; Herve *et al.*, 1993, Rangel-Barajas *et al.*, 2011). In line with this possibility, it has been reported that, in the monkey, lesion with MPTP increases the coupling of striatal D1Rs to Gαolf (Aubert *et al.*, 2005). Interestingly, during chronic treatment with L-DOPA, this change persists only in animals that develop dyskinesia (Aubert *et al.*, 2005).

A recent study performed in 6-OHDA-lesioned rats shows that dopamine depletion increases the levels of AC type V in the striatum and in the substantia nigra pars reticulata (Rangel-Barajas *et al.*, 2011), which is innervated by the MSNs of the direct pathway (cf. above). In the same study, it is also shown that this effect is maintained in dyskinetic but not in non-dyskinetic animals (Rangel-Barajas *et al.*, 2011).

Taken together these observations indicate that dopamine depletion is associated with increased D1R-mediated activation of cAMP signaling, produced by over-expression of Gαolf and AC type V. They also suggest that LID is associated with augmented coupling of D1Rs to Gαolf and to enhanced levels of AC type V, in the MSNs of the direct, striatonigral pathway. The involvement of elevated cAMP signaling in dyskinesia is supported by the observation that, in 6-OHDA-lesioned rats, striatal infusion of the cAMP-dependent protein kinase (PKA) inhibitor, Rp-cAMPS, attenuates LID (Lebel *et al.*, 2010). This finding indicates the importance of identifying molecular changes occurring downstream of cAMP/PKA and potentially implicated in LID.

### A. Pathological Anchoring of D1R at the Membrane

As mentioned in the previous section, dopamine depletion is not sufficient *per se* to affect the levels of D1Rs cf. (Aubert *et al.*, 2005; Breese *et al.*, 1987; Hurley *et al.*, 2001; Joyce, 1991; Marshall *et al.*, 1989; Pimoule *et al.*, 1985; Savasta *et al.*, 1988; Shinotoh *et al.*, 1993). However, loss of dopamine in combination with chronic administration of L-DOPA, which leads to the development of LID, has been shown to increase the expression and modify the subcellular distribution of D1Rs. Indeed, in dyskinetic rats and monkeys, D1R is more abundant at the plasma membrane compared with control animals (Berthet *et al.*, 2009; Guigoni *et al.*, 2007) suggesting that LID is associated with deficiencies in D1R desensitization and trafficking (Bezard *et al.*, 2005; Guigoni et al., 2007). This idea is supported by studies showing that GPCR kinases (GRK), which start the process of homologous desensitization by phosphorylating the receptor, and arrestins, which bind to the phosphorylated receptor initiating receptor internalization (Shenoy and Lefkowitz, 2003), are both downregulated in comparison to D1Rs, in the striatum of dyskinetic monkeys (Bezard *et al.*, 2005) and rats (Ahmed *et al.*, 2008). However, it should be noted that, although impaired, D1R internalization is still possible. Thus, in dyskinetic animals, D1Rs retain their ability to internalize after stimulation by a D1R agonist (Berthet *et al.*, 2009). Moreover, this phenomenon is not limited to D1R, as both NMDA and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors are recruited at the membrane in dyskinetic monkeys (see below) (Silverdale *et al.*, 2010).

The above findings suggest that, in LID, D1Rs are preferentially anchored at the plasma membrane when bound to dopamine (the natural non-specific DA receptor ligand) and that interventions aiming at counteracting this phenomenon may reduce dyskinesia. In line with this idea, recent findings have shown that dyskinesia is reduced by promoting the expression of specific components of the homologous desensitization machinery, such as GRK. It was found that lentiviral-mediated overexpression of GRK6 reduced behavioral sensitization and L-DOPA-induced

abnormal involuntary movements (a surrogate marker of dyskinesia in rodents) in the 6-OHDA rat model. Importantly, the same intervention was also able to alleviate LID in MPTP-lesioned monkeys (Ahmed *et al.*, 2010). Interestingly, the reverse effect, that is worsening of LID, was observed when the expression of GRK6 was further downregulated by transfection with a GRK6 miRNA. GRK6 overexpression was able to induce specifically D1R internalization without affecting other GPCRs potentially implicated in dyskinesia such as the D2R and the type 5 metabotropic glutamate receptor (mGluR5) (see below). Thus, GRK6-mediated attenuation of sensitized D1R-mediated transmission was clearly highlighted as the key factor leading to decreased LID severity (Ahmed *et al.*, 2010).

### B. Dopamine D3 Receptors in LID

The dopamine D3 receptor (D3R) represents an interesting target for the development of anti-dyskinetic drugs. D3Rs are expressed in striatonigral MSNs (Bordet *et al.*, 2000), where they exert a synergistic effect on D1Rs through direct intramembrane interaction (Fiorentini *et al.*, 2008; Marcellino *et al.*, 2008). In view of the involvement in dyskinesia of increased D1R transmission, disruption of such D1R-D3R cross-talk may affect LID. Studies in rodents and non-human primates have demonstrated that dyskinesia is accompanied by increased expression of D3Rs (Bezard *et al.*, 2003; Bordet *et al.*, 1997; Gross *et al.*, 2003; Hurley *et al.*, 1996a, 1996b). This effect occurs mainly in the prodynorphin expressing MSNs of the direct pathway and depends on the activation of D1Rs (Bordet *et al.*, 2000). The increase in D3R expression is triggered by enhanced levels of brain-derived neurotrophic factor (BDNF) (Guillin *et al.*, 2001). BDNF, in turn, is activated via D1R-mediated activation of CREB (Carlezon *et al.*, 2005), whose levels are increased in LID (Guigoni *et al.*, 2005; Oh *et al.*, 2003).

Taken together, the above observations suggest that enhanced levels of D3Rs may participate to the development of LID by further exacerbating sensitized D1R-mediated transmission. Activation of D3Rs may exert this effect by increasing the anchoring of D1Rs at the plasma membrane. In support of this possibility, co-treatment with L-DOPA and the D3R antagonist ST 198 restores normal levels of membrane-bound D1R in dyskinetic animals (Berthet *et al.*, 2009) and counteracts dyskinesia in rodent and non-human primate models of PD (Bezard *et al.*, 2003; Visanji *et al.*, 2009).

### C. Abnormal Activation of DARPP-32

One of the major targets of PKA in MSNs is the dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) (Walaas and Greengard, 1984; Walaas *et al.*, 1983). PKA catalyzes the phosphorylation of DARPP-32 at

a Thr in position 34. This, in turn, converts DARPP-32 into an inhibitor of protein phosphatase-1 (PP-1) (Hemmings *et al.*, 1984), thereby suppressing dephosphorylation of other downstream effector proteins and amplifying PKA-mediated responses (Greengard, 2001). Through this mechanism, DARPP-32 plays a critical role in D1R-mediated transmission in the basal ganglia (Fienberg *et al.*, 1998; Greengard, 2001).

Studies performed in various animal models show that depletion of dopamine results in a remarkable increase in the ability of L-DOPA to promote DARPP-32 phosphorylation at Thr34 (Santini *et al.*, 2007, 2010). This effect, which is a direct consequence of sensitized D1R/cAMP/PKA signaling, persists during chronic administration of L-DOPA only in animals that develop dyskinesia (Lebel *et al.*, 2010, Picconi *et al.*, 2003; Santini *et al.*, 2007, 2010). In line with these observations, it has been shown that LID is attenuated in DARPP-32 knock out mice (Santini *et al.*, 2007). Interestingly, LID is also reduced following cell-specific inactivation of DARPP-32 in the MSNs of the direct pathway whereas it is maintained in mice lacking DARPP-32 in the MSNs of the indirect pathway (Bateup *et al.*, 2010). Taken together these studies indicate the importance of enhanced cAMP/PKA/DARPP-32 signaling in LID and point to the MSNs of the direct pathway as a key neuronal substrate implicated in this condition.

The persistent hyper-phosphorylation of DARPP-32 associated with LID may have profound repercussions on the state of excitability of MSNs. High-frequency stimulation of cortical afferents to striatal MSNs is known to increase synaptic efficiency by inducing long-term potentiation (LTP) (Calabresi *et al.*, 1992). This phenomenon requires dopaminergic innervation and is abolished by lesion with 6-OHDA (Centonze *et al.*, 1999; Picconi *et al.*, 2003). LTP can be reversed by low frequency stimulation, which re-establishes normal level of excitability at cortico-striatal synapses (Picconi *et al.*, 2003). This phenomenon, called depotentiation, is thought to prevent the generation of aberrant motor patterns by re-instating normal levels of striatal synaptic efficiency and "erasing" non-essential motor information. Experiments performed in 6-OHDA-lesioned rats showed that LID is accompanied by loss of depotentiation in the striatum. Interestingly, cortico-striatal depotentiation is abolished by blockade of PP-1 (Picconi *et al.*, 2003). It is, therefore, possible that the absence of depotentiation associated with LID is caused by specific changes occurring along the D1R/PKA signaling pathway leading to abnormally high levels of Thr34 phosphorylated DARPP-32 and, consequently, to inhibition of PP-1 (cf. above).

The increase in PKA/DARPP-32 signaling observed in dyskinesia leads to enhanced phosphorylation of the GluR1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor at the PKA site, Ser845 (Santini *et al.*, 2007). Phosphorylation of GluR1 at Ser845 promotes excitatory glutamatergic transmission (Banke *et al.*, 2000; Mangiavacchi and Wolf, 2004) and may participate to the block of depotentiation observed in

dyskinetic rats (Picconi *et al.*, 2003). Furthermore, in MPTP-lesioned non-human primates, dyskinesia is accompanied by augmented synaptic recruitment of AMPA receptor GluR2/3 subunits (Silverdale *et al.*, 2010). Indeed, enhanced AMPA receptor transmission appears to be implicated in dyskinesia. For example, in non-human primates LID is increased by an AMPA receptor agonist and reduced by an AMPA receptor antagonist (Konitsiotis *et al.*, 2000).

## IV. Increased ERK Signaling in LID: Transcriptional and Translational Changes

Abnormalities in synaptic plasticity, such as loss of depotentiation, may also occur in response to changes in the activity of signaling pathways involved in the regulation of protein expression. In this regard, the extracellular signal-regulated protein kinase 1 and 2 (ERK) cascade, which regulates transcriptional and translational processes (Costa-Mattioli *et al.*, 2009; Thomas and Huganir, 2004), represents a particularly interesting subject of study.

In the striatum, ERK are regulated by activation of NMDA glutamate receptors and D1Rs (Sgambato *et al.*, 1998; Valjent *et al.*, 2005) and are involved in the induction of LTP (Xie *et al.*, 2009). Depletion of dopamine confers to L-DOPA the ability to activate ERK in the striata of rodents and non-human primates (Gerfen *et al.*, 2008, Lebel *et al.* 2010, Pavon *et al.*, 2006; Santini *et al.*, 2007, 2010; Westin *et al.*, 2007). This effect, which occurs specifically in the D1R expressing MSNs of the direct pathway (Darmopil *et al.*, 2009; Santini *et al.*, 2009a), depends on DARPP-32 phosphorylation (Santini *et al.*, 2007, but see also Gerfen *et al.*, 2008) and persists in association with dyskinesia (Gerfen *et al.*, 2008; Lebel *et al.*, in press, Pavon *et al.*, 2006; Santini *et al.*, 2007; Westin *et al.*, 2007).

In the mouse, LID is associated with changes in the expression of the calcium and diacylglycerol-guanine exchange factors (CalDAG-GEF) I and II (Crittenden *et al.*, 2009) which are highly expressed in striatal MSNs and act by promoting ERK signaling via regulation of Ras family G proteins (Kawasaki *et al.*, 1998; Toki *et al.*, 2001). In particular, it has been found that the severity of dyskinesia correlates with increased expression of CalDAG-GEFII and decreased expression of CalDAG-GEFI (Crittenden *et al.*, 2009). These changes may be responsible for the abnormal activation of ERK observed in dyskinetic rodents.

The importance of ERK in the development of LID is supported by the observation that the inhibition of ERK phosphorylation reduces the severity of AIMs induced by L-DOPA in the mouse (Santini *et al.*, 2007) and in the rat (Schuster *et al.*, 2008). Moreover, it has been shown that genetic inactivation, or downregulation of Ras-guanyl nucleotide releasing factor 1 (Ras-GRF1), a brain

specific activator of the ERK cascade (Farnsworth *et al.*, 1995), attenuates dyskinesia in mice and non-human primates (Fasano *et al.*, 2010).

### A. Modifications of ERK Nuclear Targets

Studies in 6-OHDA lesioned rodents showed that LID is associated with increased phosphorylation of the mitogen and stress-activated kinase 1 (MSK1) (Santini *et al.*, 2007; Westin *et al.*, 2007), a nuclear target of ERK. MSK1 phosphorylates the transcription factor cAMP response element binding protein (CREB) (Sgambato *et al.*, 1998) and increased CREB phosphorylation has been found to correlate with dyskinesia (Oh *et al.*, 2003). However, the significance of this change remains to be fully understood, since downregulation of CREB with an anti-sense oligonucleotide does not affect the ability of L-DOPA to induce dyskinesia (Andersson *et al.*, 2001).

LID is also accompanied by increased phosphorylation of histone H3 (Darmopil *et al.*, 2009; Santini *et al.*, 2007, 2009a) which is another important nuclear target of the ERK/MSK1 signaling cascade (Davie, 2003). In addition, a comparative study conducted in mouse and non-human primate models indicates that LID is associated with deacetylation of histone H4 (Nicholas *et al.*, 2008). The overall impact of these chromatin modifications on gene expression and, ultimately, on dyskinesia needs to be further elucidated, particularly considering that phosphorylation of histone H3 at Ser10 is involved in transcriptional activation, whereas histone deacetylation is generally regarded as a repressive mechanism (Berger, 2007; Nowak and Corces, 2004).

### B. Changes in Immediate Early Gene Expression

Among the various genes potentially involved in the expression of LID, the immediate early gene coding for the transcription factors FosB has received particular attention. The dorsolateral striata of dyskinetic rats and monkeys contain higher levels of FosB and of its alternatively spliced isoforms, collectively named ΔFosB (Andersson *et al.*, 1999; Berton *et al.*, 2009). In rodents, increased ΔFosB expression is restricted to the MSNs of the direct pathway (Andersson *et al.*, 1999; Darmopil *et al.*, 2009) where activation of ERK is also occurring (Darmopil *et al.*, 2009; Santini *et al.*, 2009a). Indeed, ERK activation has been involved in the increase in *fosB* expression produced by dopaminomimetic drugs such as cocaine (Zhang *et al.*, 2004). Enhanced levels of FosB appear to be causally related to the development of dyskinesia. Thus, striatal injection of a *fosB* anti-sense oligonucleotide reduces LID (Andersson *et al.*, 1999). A similar effect has been recently observed, in the macaque, following

viral overexpression of a dominant negative of ΔFosB (Berton et al., 2009). Conversely, in the rat, viral vector-induced overexpression of ΔFosB exacerbates LID (Cao *et al.*, 2010).

One question raised by the above findings is the identifications of specific genes regulated by ΔFosB and implicated in LID. The increase in FosB-like immunoreactivity associated with dyskinesia is involved in the up-regulation of mRNA coding for the opioid peptide, prodynorphin, which is selectively expressed by the MSNs of the direct pathway (Andersson *et al.*, 1999). However, a precise assessment of the role played by increased opiod transmission in dyskinesia is complicated by contrasting data on the effects of opioid receptor antagonists on LID (Samadi *et al.*, 2006). Further studies will be necessary to fully characterize the significance of this and other FosB-dependent effects for the development and/or expression of LID.

Another transcription factor implicated in dyskinesia is that encoded by the immediate early gene *zif268* (or *NGFI-A/krox24/egr1*). The ability of L-DOPA to increase *zif268* mRNA in MSNs is dramatically enhanced in both striatopallidal and striatonigral neurons, following dopamine depletion. Interestingly, repeated administration of L-DOPA to 6-OHDA-lesioned rats normalizes the levels of *zif268* mRNA in the neurons of the indirect pathway, but not in those of the direct pathway (Carta *et al.*, 2005). The lack of normalization of *zif268* expression in the MSNs of the direct pathway may be due to the persistent activation of ERK observed in these cells in association with dyskinesia (Gerfen *et al.*, 2008; Lebel *et al.*, in press, Pavon *et al.*, 2006; Santini *et al.*, 2007; Westin *et al.*, 2007).

Zif268 promotes the expression of the activity-regulated cytoskeletal-associated protein *arc* (or *arg3.1*) (Li *et al.*, 2005), an immediate early gene involved in syaptic plasticity (Bramham *et al.*, 2008). Interestingly, dyskinesia is accompanied by increased *arc* expression in the MSNs of the direct pathway (Sgambato-Faure *et al.*, 2005). In the hippocampus, zif268-induced expression of *arc* has been implicated in the induction of the late phase of LTP (Li *et al.*, 2005). Therefore, it is possible that the persistent overexpression of *zif268* and *arc* is involved in the suppression of depotentiation at corticostriatal synapses, observed in association with LID (cf. above) (Picconi *et al.*, 2003).

### C. Involvement of Mammalian Target of Rapamycin in LID

Recent evidence indicates that the activation of ERK associated with LID promotes signaling along the mammalian target of rapamycin complex 1 (mTORC1) cascade (Santini *et al.*, 2009b). Administration of L-DOPA to 6-OHDA-lesioned mice increases the phosphorylation of several effectors of

mTORC1 including the initiation factor 4E-binding protein, the p70 ribosomal S6 kinases, and the ribosomal protein S6 (Santini *et al.*, 2009b). Collectively, these changes, which are mediated via activation of D1Rs in the MSNs of the direct pathway (Santini *et al.*, 2009b), are known to promote initiation of translation (Richter and Sonenberg, 2005; Roux *et al.*, 2007; Ruvinsky and Meyuhas, 2006) and may participate to the development of dyskinesia. In support of this possibility it has been shown that rapamycin, an allosteric inhibitor of mTORC1 (Oshiro *et al.*, 2004), reduces the development of dyskinesia when administered in combination with L-DOPA (Santini *et al.*, 2009b).

## V. Glutamate NMDA Receptors and LID

Amantadine, an antagonist at the ionotropic glutamate NMDA receptor, is currently the most efficacious treatment for LID (Blanchet *et al.*, 2003; Goetz *et al.*, 2005; Snow *et al.*, 2000; Thomas *et al.*, 2004). This points to the involvement in dyskinesia of aberrant NMDA receptor mediated transmission. Activation of D1Rs is known to potentiate NMDA currents (Flores-Hernandez *et al.*, 2002) and increase the surface expression of NMDA receptors at the post-synaptic density (Hallett *et al.*, 2006). Therefore, the sensitized transmission at D1Rs associated with LID is in line with the increase in NMDA receptor function generally associated with this condition.

Most NMDA receptors in the brain are comprised of NR1 and NR2 subunits, forming a tetrameric complex of two NR1 associated with two NR2 subunits (Furukawa *et al.*, 2005). NR2 subunits exist as four subtypes, denominated NR2A-D, each subtype being encoded by a distinct gene. NR1 exists as seven subtypes (NR1a–g) which are generated by alternative splicing from a single gene (Dingledine *et al.*, 1999).

Studies performed in 6-OHDA lesioned rats indicate that LID is accompanied by increased levels of NR2A and decreased levels of NR2B at post-synaptic level. In addition, chronic administration of L-DOPA promotes the phosphorylation of NR1 and NR2 which may result from activation of D1Rs (Dunah and Standaert, 2001; Dunah *et al.*, 2004; Hallett *et al.*, 2006; Snyder *et al.*, 1998). In the rat, dyskinesia is associated with downregulation of a complex formed by D1Rs and NMDA which is thought to desensitize NMDA receptors (Fiorentini *et al.*, 2006). This modification may be related to the increase in NMDA receptor transmission generally associated to LID.

The striata of patients affected by L-DOPA-induced motor complications contain higher numbers of binding sites for receptors composed of NR1/NR2B, whereas the binding sites for NR1/NR2A appear to be unaltered (Calon *et al.*,

2003). In line with this finding, increased expression of NR2B has been reported in the striatum and other brain regions of dyskinetic marmoset monkeys (Hurley *et al.*, 2005). However, in the same species, increased levels of NR2A, but not NR2B, have been found in association with dyskinesia (Hallett *et al.*, 2005) and a similar change has been described in striatal synapses of dyskinetic rats (Gardoni *et al.*, 2006). Moreover, disruption of the ability of NR2B receptors to interact with the membrane-associated guanylate kinase (MAGUK) protein family (which include the post-synaptic density-95 and synapse-associated proteins) results in the exacerbation of LID (Gardoni *et al.*, 2006). Altogether, these various observations indicate that NMDA receptors, and NR2B in particular, are implicated in LID. However, a clear understanding of this phenomenon is complicated by contrasting reports on the ability of NR2B selective antagonists to affect LID (Hadj Tahar *et al.*, 2004; Morissette *et al.*, 2006; Nash *et al.*, 2004; Rylander *et al.*, 2009; Wessell *et al.*, 2004).

## VI. mGluR5

The mGluR5 is abundantly expressed in striatal MSNs (Kerner *et al.*, 1997; Testa *et al.*, 1994). Studies performed in parkinsonian patients and MPTP-lesioned monkeys showed increase mGluR5 binding in association with dyskinesia (Ouattara *et al.*, 2009; Samadi *et al.*, 2008). Most importantly, pharmacological blockade of mGluR5 has been found to reduce LID in rodent and non-human primate models (Dekundy *et al.*, 2006; Johnston *et al.*, 2010; Levandis *et al.*, 2008; Mela *et al.*, 2007; Morin *et al.*, 2010; Rylander *et al.*, 2010a; Yamamoto and Soghomonian, 2009). Electrophysiological studies indicate that mGluR5 agonists potentiate the excitatory NMDA response in MSNs (Pisani *et al.*, 2001) suggesting that blockade of mGluR5 may exert anti-dyskinetic action by counteracting the increase in NMDA receptor transmission associated with LID (cf. above).

## VII. Controlling Dyskinesia by Acting on the MSNs of the Indirect Pathway

Considerable evidence has been collected pointing to a critical involvement in LID of aberrant signaling at the level of the D1R-expressing MSNs of the direct pathway. Much less is known about parallel abnormalities occurring in the MSNs of the striatopallidal indirect pathway, which express selectively D2Rs. Nevertheless, a number of observations indicate that dyskinesia can be controlled by specific interventions focused on this neuronal population.

Previous work has shown that D2R agonists induce behavioral sensitization and dyskinesia in primed animals, albeit to a lesser extent as compared to D1R agonists (Calon *et al.*, 1999; Carta *et al.*, 2008, 2010; Kumar *et al.*, 2009; Pearce *et al.*, 1998; Rascol *et al.*, 2000, 2001; Smith *et al.*, 2006; Stockwell *et al.*, 2008). In dopamine-depleted animals, D2R distribution and expression are not affected by chronic treatment with L-DOPA (Aubert *et al.*, 2005; Guigoni *et al.*, 2007). Therefore, it is not surprising that a considerable part of the evidence in support of the involvement of D2Rs in LID is indirect and comes from studies on specific components of the signal transduction machinery connected to these receptors.

### A. Acting on the Regulatory GPCR signaling protein 9-2

The regulatory GPCR signaling protein 9-2 (RGS9-2) is a striatal-enriched GTPase (Rahman *et al.*, 1999) which reduces D2R-mediated transmission by accelerating the inactivation of the Gαi/o subunit coupled to D2Rs (see above) (Rahman *et al.*, 2003). RGS9-2 knock out mice show a higher propensity to develop severe LID suggesting that RGS9-2 may exert a protective action against this condition (Gold *et al.*, 2007). In keeping with this possibility, studies in animal models of LID (Gold *et al.*, 2007) and in L-DOPA-exposed PD patients (Tekumalla *et al.*, 2001) suggests that increased RGS9-2 expression may represent an adaptive response to counteract sensitized D2R transmission developed in response to dopamine depletion. Such a compensatory adaptation is *per se* insufficient to effectively reduce dyskinesia as MPTP-lesioned monkeys and L-DOPA-treated PD patients do develop LID. However, if potentiated using viral vectors, increased RGS9-2 expression is able to reduce the severity of LID in 6-OHDA-lesioned rats and MPTP-treated monkeys (Gold *et al.*, 2007), thereby unmasking the importance of exaggerated D2Rs transmission in LID. This positive effect is counterbalanced by the concomitant abolishment of the anti-parkinsonian action of ropinirole (a D2/D3R agonist) (Gold *et al.*, 2007), an observation that clearly highlights the complex role of the D2R in PD and dyskinesia.

### B. Cav1.3 L-Type $Ca^{2+}$ Channels in PD and LID

Profound plastic changes affect striatal MSNs during the progressive loss of DA input (Arbuthnott *et al.*, 1998; Ingham *et al.*, 1998; Neely *et al.*, 2007). It has been demonstrated that, in experimental rodent models of PD, the MSNs of the indirect pathway lose a significant fraction of dendritic spines and glutamatergic synapses (Day *et al.*, 2006). This loss of connectivity is triggered by a dysregulation of Cav1.3 L-type $Ca^{2+}$ channels. Reduced spines and synaptic connectivity is likely to alter information flow through the striatum and the rest of the basal

ganglia and may therefore participate in the development of adverse events related to L-DOPA therapy. Interestingly, in 6-OHDA-lesioned rats, administration of isradipine, a Cav1.2-1.3 L-type $Ca^{2+}$ channel antagonist, reduces in a dose-dependent manner L-DOPA-induced rotational behavior and AIMs (Schuster *et al.*, 2009). In addition, isradipine prevents the loss of spines induced by 6-OHDA and normalizes pre-proenkephalin-A mRNA expression in the MSNs of the indirect pathway. Involuntary movements are not reduced when isradipine treatment is started concomitantly with L-DOPA (Schuster *et al.*, 2009). These results suggest that blocking Cav1.2–1.3 L-type $Ca^{2+}$ channels, and thus dendritic spine loss on striatopallidal MSNs, represents a treatment option to prevent LID.

### C. Adenosine A2A Receptors

The MSNs of the indirect pathway are highly and selectively enriched in the A2A type of receptor for the neuromodulator, adenosine (Fink *et al.*, 1992; Schiffmann *et al.*, 1991). Activation of A2A receptors leads to Gαolf-mediated stimulation of AC and increased cAMP-signaling (Corvol *et al.*, 2001; Fredholm, 1977) which opposes the inhibitory action exerted on this pathway by D2Rs (Stoof and Kebabian, 1981). In line with this notion, antagonists at A2A receptors such as KW-6002 (istradefylline), have been proposed to relieve the symptoms of PD by promoting dopaminergic transmission (Morelli *et al.*, 2007). This effect is exerted without producing any apparent motor complication. The anti-parkinsonian properties of A2A receptor antagonists confer to these drugs the ability of potentiating the therapeutic efficacy of low doses of L-DOPA. This would indirectly counteract or delay the onset of LID whose emergence is hastened by high L-DOPA dosage.

A2A receptor antagonists may also exert a direct anti-dyskinetic effect. This idea rests for the most part on the observation that, in MPTP-lesioned non-human primates, the administration of istradefylline prevents dyskinesia induced by the dopaminergic agonist, apomorphine (Bibbiani *et al.*, 2003). Moreover, LID is reduced in mice in which A2A receptors are selectively inactivated in the forebrain (Xiao *et al.*, 2006). In contrast, a study performed in the rat did not report any effect of A2A receptor antagonists on dyskinesia (Lundblad *et al.*, 2003).

The mechanisms underlying the potential anti-dyskinetic action of A2A receptor antagonists remain to be elucidated. Emerging evidence indicates that A2A receptors are present not only post-synaptically, at the level of the MSNs of the indirect pathway, but also pre-synaptically on corticostriatal terminals, where they promote the release of glutamate (Ciruela *et al.*, 2006; Quiroz *et al.*, 2009). Therefore A2A receptor antagonists may counteract dyskinesia by inhibiting excessive glutamatergic transmission which is thought to be implicated in LID.

### VIII. Cannabinoid CB1 Receptors

The striatum is highly enriched in type 1 cannabinoid (CB1) receptors (Herkenham *et al.*, 1990), which are expressed in both populations of projection MSNs (Hohmann and Herkenham, 2000). CB1 receptors reduce glutamatergic transmission and MSNs excitability by acting on corticostriatal terminals (Brown *et al.*, 2003; Gerdeman and Lovinger, 2001), thereby suggesting a potential use of CB1 receptor agonists in the treatment of dyskinesia (cf. above). This possibility is supported by several studies showing that WIN 55,212-2 and nabilone, two CB1 receptor agonists, reduce LID in rats and non-human primates, respectively (Ferrer *et al.*, 2003; Fox *et al.*, 2002; Morgese *et al.*, 2007; Sieradzan *et al.*, 2001). It should be noted, however, that in a study conducted using MPTP-lesioned marmoset monkeys a similar anti-dyskinetic effect has been found in response to the administration of the CB1 receptor antagonist, rimonabant (van der Stelt *et al.*, 2005, but see also Cao *et al.*, 2007).

The ability of CB1 receptor agonists to reduce dyskinesia has prompted the analysis of the effects produced on LID by drugs that interfere with the metabolism of endocannabinoids which include arachidonyl-ethanolamide or anandamide. CB1 receptors can be activated through the administration of URB597, an inhibitor of the fatty acid amide hydrolase (FAAH), which catabolizes anandamide (Piomelli *et al.*, 2006). The increase in anandamide levels produced by FAAH inhibition activates not only CB1 receptors but also transient receptor potential vanilloid type-1 (TRPV1) receptors (De Petrocellis *et al.*, 2001; Ross, 2003). Interestingly, the anti-dyskinetic effect produced by anandamide-mediated activation of CB1 receptors is prevented by concomitant stimulation of TRPV1 receptors (Morgese *et al.*, 2007). Thus, URB597 effectively reduces LID only when combined with the TRPV1 receptor antagonist, capsazepine (Morgese *et al.*, 2007). TRPV1 receptors are expressed in the basal ganglia (Cristino *et al.*, 2006; Mezey *et al.*, 2000) and may therefore represent a further target for the development of anti-dyskinetic compounds.

### IX. Pre-Synaptic Mechanisms: Serotonin Receptors

It is generally assumed that, in the early stages of PD, L-DOPA is taken up into spared dopaminergic neurons and terminals, where it is converted to dopamine, stored into synaptic vesicles and released in a physiologically regulated manner (Cenci and Lundblad, 2006). As the dopaminergic degeneration progresses, fewer and fewer dopamine terminals can contribute to the conversion of peripherally

administered L-DOPA. In this situation, other neuronal and non-neuronal cell types have been suggested to play a role in dopamine production (Melamed *et al.*, 1980). Among these, the serotonin neurons represent an interesting element because they express aromatic amino acid decarboxylase (AADC) and vesicular monoamine transporter 2 (VMAT2), which are responsible for conversion of L-DOPA to dopamine and storage of dopamine into synaptic vesicles, respectively (Arai *et al.*, 1995).

Several studies have shown that the serotonin neurons have the capacity to store and release dopamine after peripheral administration of L-DOPA, both *in vivo* and *in vitro* (Ng *et al.*, 1970, 1972). Tanaka and co-workers showed that lesion of the serotonin system by intraventricular administration of the specific toxin 5,7-dihydroxytryptamine (5,7-DHT) reduced L-DOPA-derived extracellular dopamine by about 80% in hemiparkinsonian rats (Tanaka *et al.*, 1999). A similar reduction in extracellular striatal DA level was also obtained following co-administration of the 5-$HT_{1A}$ agonist (±)-8-OH-DPAT with L-DOPA (Kannari *et al.*, 2001). Carta and co-workers have elegantly demonstrated in a series of pivotal papers a causal link between the dopamine released from the serotonin neurons and the appearance of AIMs and LID (Carta *et al.*, 2007; Muñoz *et al.*, 2008). In these experiments, removal of the serotonin innervation by intraventricular injection of 5,7-DHT, or pharmacological silencing of the release from these neurons by a combination of 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor agonists, resulted in a near-complete suppression of LID in 6-OHDA-lesioned rats (Carta *et al.*, 2007). In addition, serotonin neuron transplants increased the pro-dyskinetic effect of L-DOPA by providing a 2–3-fold increase in the serotonin innervation of the host striatum and thus a possible additional source of dysregulated dopamine release (Carlsson *et al.*, 2007). It has also been shown that L-DOPA treatment *per se* induces sprouting of serotonin axon terminals in the striatum, thereby further exacerbating unregulated dopamine release (Rylander *et al.*, 2010b).

The involvement of dysfunctional serotonin function in LID is further indicated by the observation that the levels of 5-$HT_{1B}$ receptors and of their adaptor protein, p11, are increased by L-DOPA in the striata of 6-OHDA lesioned rodents (Zhang *et al.*, 2008). These effects are mediated via D1Rs and appear to be implicated in the development of LID, since treatment with the selective 5-$HT_{1B}$ receptor agonist, CP94253, reduced L-DOPA-induced abnormal involuntary movements (Carta *et al.*, 2008; Zhang et al., 2008) and this effect was abolished in p11 knock out mice (Zhang *et al.*, 2008).

These observations suggest that the dopamine released from serotonin terminals is the main pre-synaptic determinant of LID in the rodent PD model. They also indicate that 5-$HT_{1A}$ and 5-$HT_{1B}$ agonists, particularly in combination, may be employed for the treatment of LID. Thus, in the MPTP-treated macaque model of PD, a comparison between stand-alone and combined treatments with the 5-$HT_{1A}$ and 5-$HT_{1B}$ agonists demonstrated a

potent synergistic effect between these drugs in their ability to dampen LID (Muñoz *et al.*, 2008). Sub-threshold doses of 5-$HT_{1A}$ or 5-$HT_{1B}$ agonists, which individually produced no effect, reduced the abnormal involuntary movements by up to 80% when administered in combination, without affecting the anti-parkinsonian properties of L-DOPA (Muñoz *et al.*, 2008). Others have since shown that serotoninergic neurons may be responsible as well for extrastriatal release of dopamine, a likely contributing factor to LID (Navailles *et al.*, 2010, 2011).

## X. Conclusions

The use of simple rodent models in combination with more advanced non-human primate models has spurred tremendous progress toward the identification of the molecular determinants of LID and its neuronal substrates. A large amount of data indicate that this condition is linked to the sensitization developed by dopaminergic receptors, particularly D1Rs, in response to the loss of dopamine associated with PD. This phenomenon results in a dramatic increase in the ability of L-DOPA to affect not only the cAMP/PKA/DARPP-32 cascade, which is typically coupled to activation of D1Rs but also the ERK and the mTORC1 cascades.

The emerging picture suggests that the intermittent and persistent activation of these signal transduction pathways produced by L-DOPA ultimately results in the permanent modification of the functional features of striatal MSNs such as the inability to control excessive synaptic potentiation (i.e., loss of depotentiation). It still remains to be determined what are the specific signaling components responsible for "locking" corticostriatal synapses in an excited state and what is the relevance of this phenomenon in the generation of dysfunctional motor behavior.

Increased understanding of the molecular abnormalities implicated in LID has led to the design of novel therapeutic strategies, in addition to those based on the use of drugs acting at receptor level (i.e., glutamate receptor antagonists, A2AR antagonists, CB1 receptor agonists, etc.). For instance, the dyskinetic effect produced by increased dopaminergic transmission has been reduced by viral vector-mediated overexpression of proteins (i.e., GRK6 and RGS9-2) involved in D1R and D2R desensitization. LID is also counteracted, both in rodents and non-human primates, through inhibition of ERK and mTORC1 signaling.

In conclusion, the study of the molecular mechanisms of LID represents a successful example of translational approach, in which information obtained at molecular and cellular level using mouse and rat models is tested in more advanced non-human primate models, ultimately providing essential knowledge for the design of more effective clinical strategies.

## Acknowledgments

The authors were supported by grants from the Swedish Research Council (GF), the Swedish Brain Foundation (GF), the Michael J. Fox Foundation for Parkinson Research (GF and EB), MAPKinDYSK (EB), TRAFinLID (EB), and MCHPRIMAPARK (EB) Agence Nationale de la Recherche grants.

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# NEW APPROACHES TO THERAPY

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L-DOPA-induced dyskinesia (LID) is a major complication of the treatment of Parkinson's disease (PD). LID comprises two major components, the priming process responsible for its onset and the expression of involuntary movements that underlies its clinical manifestation. The mechanisms responsible for these components are partially understood and their biochemical basis is being unraveled but avoidance and treatment remain an issue. In this chapter, we review what is known about the involvement of dopaminergic systems in LID and the way in which dopaminergic therapy can be used to avoid the onset of LID or to reverse or suppress involuntary movements once these have been established. The involvement of specific dopamine receptor subtypes, continuous dopaminergic stimulation (CDS) and continuous drug delivery (CDD) is reviewed. However, a major role is emerging in the avoidance and suppression of LID through the use of nondopaminergic mechanisms and we consider the present and future use of glutamatergic drugs, serotoninergic agents, adenosine antagonists and others as a means of improving therapy. There is compelling basic science supporting a role for nondopaminergic approaches to LID but at the moment the translational benefit to PD is not being achieved as predicted. There needs to be further consideration of why this is the case and how in future, both experimental models of dyskinesia and clinical trial design can be optimized to ensure success.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00005-5





0074-7742/10 $35.00

## I. Introduction

L-DOPA-induced dyskinesia (LID) remains a common side effect of the treatment of Parkinson's disease (PD) affecting between 30% and 40% of the patient population (Schrag and Quinn, 2000). Although LID is mild and nontroublesome in the majority of affected individuals, it becomes severe and troublesome and treatment limiting in significant patient numbers. The clinical phenomenology and assessment are well described but the underlying causes of dyskinesia remain uncertain. There appear to be two important components—the induction of dyskinesia that leads to the persistent expression of dyskinesia that is commonly referred to as the priming phenomena and the subsequent expression of dyskinesia in response to every dose of dopaminergic medication (Jenner, 2008). From a mechanistic perspective, these appear to be distinct events. Priming for dyskinesia again appears to be related to two major considerations—first, the risk of developing dyskinesia increases with the extent of nigral cell degeneration and the more advanced stages of the illness, both of which lower the threshold for dyskinesia induction and the degree of L-DOPA exposure required. Second, the nature of early drug exposure appears to determine the risk of dyskinesia induction with an apparent difference between a high risk associated with L-DOPA use compared to the effects of dopamine agonists. This might reflect differences in their duration of action and the nature of postsynaptic dopamine receptor stimulation or simply differences in their pharmacology. Once dyskinesia is established, it is generally held that the same pattern of involuntary movements is evoked by all dopaminergic treatments—but this view will be challenged in this chapter.

The treatment of dyskinesia remains a significant problem and it is currently an unmet need in the therapeutic approaches taken to PD (Jankovic and Stacy, 2007). The usual avenues are to reduce dopaminergic medication, to attempt to deliver drugs more continuously by subcutaneous or intraduodenal infusion, to add in treatment with the NMDA antagonist amantadine or to resort to a surgical approach, most notably implantation of electrodes into the overactive subthalamic nucleus (deep brain stimulation (DBS)). However, none of the pharmacological strategies employed leads to the effective suppression of dyskinesia without a loss of control of motor symptomatology or the onset of adverse events and infusions, and surgical approaches are invasive and of limited application to the whole patient population. In addition, while dyskinesia can be suppressed, there appears to be little that can be done to reverse the underlying priming process. This chapter reviews novel therapeutic approaches that are currently undergoing preclinical and clinical evaluation for the prevention and treatment of LID in PD and many of which target nondopaminergic neuronal systems.

The investigation of novel experimental approaches to dyskinesia has been underpinned by the availability of effective animal models of PD where LID can be

induced and expressed (Duty and Jenner, 2011). These are most notably, the L-DOPA treated 6-OHDA-lesioned rat expressing abnormal involuntary movements (AIMs) and the L-DOPA treated MPTP-treated primate showing chorea, dystonia, and athetosis (Pearce *et al.*, 1995; Winkler *et al.*, 2002). In these models, as well as through the use of postmortem tissues from PD, it has been possible to explore the nature of changes that occur in efferent and afferent pathways in the cortico-basal ganglia-thalamic loop that controls voluntary movement and in which dyskinesia is thought to originate. These studies have identified numerous neuronal receptor targets that can potentially improve motor function in PD and prevent the onset and expression of dyskinesia. These include glutamate, opioid, cannabinoid, cholinergic, adenosine, histamine, and serotonin receptors (Fox *et al.*, 2008). In addition, intracellular signaling cascades have been identified that offer the opportunity to intervene in processes linked to the priming for dyskinesia as well as its expression (Bezard *et al.*, 2005; Cenci and Konradi, 2010; Jenner, 2008; Santini *et al.*, 2010). However, experimental models of PD are focused on the primary loss of the nigrostriatal pathway that occurs in PD, and they do not reflect the more widespread pathology and adaptive changes that occur in brain in PD. These also might contribute to the risk of developing dyskinesia and to its subsequent avoidance and suppression. Consequently, there is increasing interest in nondopaminergic inputs to basal ganglia, notably serotoninergic and noradrenergic pathways. As a consequence, an extensive range of new therapeutic moieties are under investigation and form the focus for this review.

The unmet therapeutic need in treating LID in PD comes from several directions and these form the basis of the subsequent sections. The requirement is for

1) therapies to stop the development of LID;
2) therapies to suppress LID, once it has developed while continuing to use L-DOPA;
3) therapies to reduce/avoid LID involving reducing L-DOPA or switching to other medications; and
4) therapies to reduce priming for LID.

We will discuss all of these issues but divide the available information in to that relating to dopaminergic therapy and that which relates to nondopaminergic approaches to treatment.

## II. Factors that Control the Priming and Expression of LID

The two major factors that influence the development of dyskinesia are, first the nature of drug exposure and second the severity of dopaminergic cell loss (Schneider, 1989; Kuoppamaki *et al.*, 2007). In particular, the more intermittent

the dopaminergic stimulation with mixed activation of D1/D2 receptors and the more severe the dopaminergic degeneration, the more rapidly dyskinesia will develop once L-DOPA treatment is initiated. It appears likely that the extent of nigral cell loss changes the sensitivity of basal ganglia for priming by dopaminergic agents as repeated treatment with high-dose L-DOPA can lead to the development of dyskinesia in normal animals (Pearce *et al.*, 2001; Togasaki *et al.*, 2001).

The neural mechanisms responsible for the expression of dyskinesia, once it has developed, likely involve abnormal neuroplasticity in the striatum and result in imbalances between the activity of medium spiny neurons of the direct and indirect striatal output pathways (Crossman, 1989; Crossman, 1990; Obeso *et al.*, 2000). The mechanisms by which intermittent dopamine replacement therapy, especially in the dopamine-depleted brain, sensitizes the striatum such that it becomes primed to elicit dyskinesia on subsequent challenges with L-DOPA, or other dopaminergic drugs, are less clear. However, in recent years, a concept has arisen that proposes that in a manner similar to the expression of dyskinesia, the "priming" process may involve an interplay between the direct and indirect pathways (Brotchie *et al.*, 2005). The role of dopaminergic therapy in dyskinesia development focuses on the direct pathway. In lesioned animals, repeated administration of L-DOPA leads to a remarkable increase of the expression of D3 dopamine receptors in neurons of the direct pathway that appears to be driven by D1 dopamine receptor activation (Bordet *et al.*, 2000). In the normal dorsal motor striatum, D3 dopamine receptors are essentially absent and their presence after priming can be considered ectopic (Bordet *et al.*, 1997). These abnormal D3 receptors may play a role in both the process of priming and expression of dyskinesia (Bezard *et al.*, 2003; Hsu *et al.*, 2004; Visanji *et al.*, 2009). This suggests that it might be useful to employ D3 dopamine receptor antagonists as a means to reduce the development of dyskinesia, for instance, by using them in combination with L-DOPA from the outset of treatment. Alternatively, this concept highlights the potential value of developing dopamine replacement therapy that avoids stimulation of D1-like or D3 receptors and/ or provides continuous stimulation of dopaminergic receptors. Both approaches are discussed below.

The role of denervation focuses attention on the role of the indirect output pathway. Understanding how dopamine depletion sensitizes to the priming effects of intermittent dopaminergic stimulation, and increases the propensity of dopaminergic treatments to lead to dyskinesia development, requires some discussion. In untreated PD, the indirect pathway bears the brunt of abnormalities, at least in terms of basic firing rates and patterns. This leads to increased signaling in the indirect pathway involving both the corticostriatal glutamatergic pathway and A2a adenosine receptors (Brotchie, 2005). This is demonstrated by the ability of a range of glutamate receptor antagonists and A2a adenosine antagonists to exert antiparkinsonian activity. As discussed below, the abnormal regulation of glutamatergic and adenosinergic control of the indirect pathway may also contribute to

the priming process. In contrast, their role in the development of dyskinesia is poorly understand and based largely on empirical evidence. This shows reduced development of dyskinesia following combined treatment with an adenosine or glutamate antagonist and L-DOPA. Likewise, it is not clear how changes in the direct pathway, initiated by D1 receptor stimulation, interact with changes in the indirect pathway and underlie priming. Such interplay between the direct and indirect pathway may involve signaling between recurrent collaterals of medium spiny neurons of the direct and indirect pathway or might involve additional neuromodulatory processes. For example, a D3-BDNF cascade has the potential to modulate corticostriatal inputs, including those to the indirect pathway (Guillin *et al.*, 2003). Notwithstanding the paucity of mechanistic explanations, the data available at present do support the development of A2a adenosine and glutamatergic antagonists as adjuncts to dopamine replacement.

## III. Modifying LID Through Dopaminergic Approaches

The requirement of priming for LID for intermittent stimulation of both D1 and D2 dopamine receptors highlights approaches which have been developed through preclinical models of PD and that have had a significant impact on clinical practice. These involve the introduction of dopamine agonists that are selective for D2-like receptors, continuous dopaminergic stimulation (CDS) using longer acting dopamine agonists and more recently, continuous drug delivery (CDD) (Jenner, 2009). These have led to a move away from initiating treatment of PD with classical orally administered formulations of L-DOPA with a peripheral aromatic amino acid decarboxylase inhibitor, such as benserazide or carbidopa to employing D2/D3 selective dopamine agonists and to strategies that deliver dopaminergic stimulation in a continuous, or at least less intermittent, manner (Olanow *et al.*, 2006; Olanow, 2008).

### A. Lessons from Dopamine Receptor Agonists

Selective D2/D3 dopamine receptor agonists have been available for clinical use since 1970s. Based on the concept that dopamine derived from L-DOPA-induced dyskinesia through the stimulation of both D1-like and D2-like dopamine receptors linked to the direct and indirect pathways, it was proposed that D2 agonists might alleviate motor symptoms of PD without dyskinesia induction. This was thought to involve an action on the indirect pathway through D2 receptors without activity at D1 receptors on the direct pathway so avoiding priming for dyskinesia. The concept has been widely tested in preclinical models of PD, namely

the 6-OHDA-lesioned rat and the MPTP-treated primate, and in the clinic is widely held to be true. Thus, in MPTP-treated primates, monotherapy of previously drug-naive animals with the D2 agonists ropinirole, pramipexole, pergolide, piribedil, and pardoprunox induces sustained reversal of motor deficits that was equivalent to that produced by L-DOPA (Johnston, 2010; Maratos *et al.*, 2003; Pearce *et al.*, 1998; Smith *et al.*, 2002; Tayarani-Binazir *et al.*, 2010). However, the intensity of dyskinesia at the end of the treatment period was significantly less than that produced by L-DOPA. In long-term clinical study in PD, the same appears to be true. Thus, 5 years *de novo* treatment with ropinirole was associated with a significantly lower prevalence of dyskinesia than occurred with L-DOPA (Rascol *et al.*, 2000). Likewise, 5 years *de novo* cabergoline, 2 years pramipexole, and 3 years of pergolide treatment were associated with less dyskinesia than L-DOPA treatment (Bracco *et al.*, 2004; Oertel *et al.*, 2006; Parkinson's Study Group, 2009). This success might have been expected to lead the introduction of therapy in PD to be invariably started with a D2 agonist rather than L-DOPA so removing the issue of dyskinesia induction. However, this has not happened for a combination of reasons and more recently there has been an increasing trend to return to early L-DOPA therapy. Notably, selective D2/D3 dopamine agonists have failed to demonstrate the efficacy provided by L-DOPA in early PD. In addition, the vast majority of the dyskinesias produced by L-DOPA are of the "nontroublesome" type with no difference in "troublesome" or painful involuntary movements (Parkinson's Study Group, 2009). With time, as disease advances, the agonist monotherapy invariably fails to adequately control motor symptoms, and supplementation with L-DOPA is inevitably required. This may argue that in addition to involvement in dyskinesia, agonism at D1 receptors may also contribute to antiparkinsonian activity (see later). Additional issues that complicate the use of D2/D3 agonists as first line treatment in early PD include the propensity to induce impulse control disorders, daytime somnolence and, with ergot derived dopamine agonists, such as bromocriptine, pergolide, and cabergoline, a potential to cause cardiac valve pathology and pulmonary fibrosis (Chaudhuri *et al.*, 2004). However, it is the major role played by the extent of neuronal loss in dyskinesia induction that is starting to limit dopamine agonist use. Long-term follow-up of the early agonist monotherapy studies suggests that there is no difference in final outcome for motor complications dependent on the initial treatment option (Katzenschlager *et al.*, 2008). Indeed, the "honeymoon" period for L-DOPA is reduced in later disease as less exposure to the drug is required to induce LID.

Some of the arguments used above raise the question of the role of the various dopamine receptor subtypes in the genesis of dyskinesia and in the control of motor symptoms of PD. From the outset, it is important to make it clear that dopamine receptor subtypes are not only present in the striatum but are also selectively localized in the GPe and GPi and the subthalamic nucleus as well as in substantia nigra. These receptors are innervated by collaterals from the nigrostriatal pathway

and while it is clear that this innervation is lost in PD, the role of extra-striatal dopamine receptors in the symptomatology and treatment of PD remains largely unknown. D1-like receptors (D1/D5) are clearly important in PD as a range of D1 receptor agonists are able to exert antiparkinsonian activity in 6-OHDA-lesioned rats and MPTP-treated monkeys (Jenner, 1995; Jenner, 2002; Temlett *et al.*, 1989). Limited clinical experience has also shown an antiparkinsonian effect of the D1 agonists, dihydrexidine, CY-208-243, and ABT 431 in man. What is strange considering the proposed role of D1 receptors in priming for dyskinesia, is that little seems to have done to put this to a functional test by examining the ability of D1 agonists to induce involuntary movements in previously drug-naive animals. The limited evidence available suggests that dyskinesia can be induced in MPTP-treated primates but there has been no comparison to the effects of L-DOPA or a D2 agonist in the same study. Nothing appears to be known in PD. There are some clues from the effects seen with dopamine agonists that have some D1 activity coupled to their effects on D2/D3 receptors. Again in drug-naive MPTP-treated primates, a comparison across studies shows dyskinesia is induced more intensely by apomorphine, pergolide, and piribedil than by ropinirole, pramipexole, or bromocriptine. There are also some intriguing insights into the interaction between D1 and D2 receptors in dyskinesia induction. In studies involving the repeated administration of ropinirole or piribedil, the intensity of resulting dyskinesia was low compared to comparable doses of L-DOPA but on switching to L-DOPA treatment, the first exposure to the drug-induced intense involuntary movements (Jackson *et al.*, 2007; Smith *et al.*, 2006). In contrast, switching animals with intense dyskinesia due to L-DOPA exposure, to an equivalent dose of a dopamine agonist, reduced dyskinesia intensity by half. This suggests that administration of D2/D3 dopamine agonists alone does not lead to marked dyskinesia expression but that they do prime for involuntary movements and that this is exposed when L-DOPA administration stimulates both D2-like and D1-like receptors. This synergy is also seen when using a combination of a dopamine agonist and L-DOPA (Maratos *et al.*, 2001). Treatment with ropinirole in a dose producing a maximal improvement in motor function in MPTP-treated primates resulted in some dyskinesia. Presumably, a maximal occupation of D2 receptors ensued but the addition of pulsatile L-DOPA to ropinirole treatment caused a marked increase in dyskinesia intensity and the obvious explanation would be the additional action on D1 receptors (Zubair *et al.*, 2007). All of these data suggest a role for both D1 and D2 receptors in dyskinesia induction with the D1 component necessary for the expression of the more intense involuntary movements.

As mentioned previously, the role of the D3 receptor in dyskinesia induction is also of importance. In 6-OHDA-lesioned rats, the D3 antagonist S33084 reduced the development of L-DOPA-induced sensitization of rotational behavior that reflects priming for LID (Visanji *et al.*, 2009). Moreover, this treatment also reduced markers of abnormal activity in the direct pathway that have been

traditionally associated with the development of dyskinesia, for example, an elevation of the opioid precursor, preproenkephalin-B (PPE-B). In the MPTP-treated primate, the development of dyskinesia is prevented when S33084 therapy is initiated alongside L-DOPA therapy (Visanji *et al.*, 2009). S33084 does not reduce the antiparkinsonian action of L-DOPA. Importantly, when the animals treated with a combination of S33084 and L-DOPA were subsequently challenged with L-DOPA alone, little dyskinesia was apparent suggesting that S33084 inhibited priming for dyskinesia rather than suppressing the expression of LID. Indeed, in animals that had been treated with L-DOPA alone leading to marked dyskinesia expression, administration of S33084 in combination with L-DOPA did not lead to inhibition of established involuntary movements. This finding highlights the difference in pharmacology of priming for LID compared to its expression.

### B. Lessons from CDS

The alternative view of dyskinesia induction has been that producing a physiological tonic stimulation of dopamine receptors is less likely to produce perturbations in basal ganglia function that lead to the onset of involuntary movements. This has again centered around differences between dopamine agonist drugs and L-DOPA, and their ability to induce dyskinesia in early PD and in primate and rodent models of PD. The argument has been that dopamine agonists are more likely to produce CDS than L-DOPA based on their longer plama half-life (Olanow *et al.*, 2006). Certainly, the concept is supported by the long half-life D2 agonists such as cabergoline where a plasma half-life of 65 h is associated with a low rate of development of dyskinesia in early PD compared to L-DOPA whose half-life is in the range of 1.5–2 h (Bracco, 2004). What is difficult, is to distinguish between the effects of cabergoline that derive from CDS as opposed to D2 receptor selectivity. In fact, CDS does not appear to be as rigorous a concept of how to treat PD as initially thought. Clinical evaluation of different dopamine agonists of differing half lives or duration of effect has not been undertaken in head-to-head studies so there has to be a reliance of data arising from preclinical investigations and most notably the MPTP-treated primate (Jenner, 2008; Jenner, 2009). It is certainly true that the longer acting oral dopamine agonists used clinically to treat PD produce less dyskinesia than equivalent doses of L-DOPA in this model. However, short-acting dopamine agonists, such as rotigotine administered by subcutaneous injection, also result in much less dyskinesia and overall there seems to be no correlation with plasma half-life or duration of effect (Stockwell *et al.*, 2009). This takes the argument back to the previous discussion that the difference lies in the pharmacology of L-DOPA and dopamine agonists and not in their pharmacokinetics. Once more the D1 receptor seems to be the biggest unknown in the debate over CDS. There is virtually no data on the

relative effects of short-acting and long-acting D1 agonists and the development of dyskinesia. What is clear is that tolerance develops rapidly to the repeated administration of long-acting D1 agonists and this is associated with a loss of their antiparkinsonian activity but how this relates to the role of the D1 receptor in dyskinesia induction is far from clear (Smith *et al.*, 2002b). This is important as the D1 receptor is implicated in the propensity of L-DOPA to induce dyskinesia but precisely how has not been defined. One last difficulty over CDS is whether plasma half-life defines the striatal actions of dopamine agonist drugs and the duration of receptor occupancy. For most this has not been studied but there are interesting PET investigations with the D2 partial agonist pardoprunox. While its plasma half-life of 1–3 h suggests a short-acting drug, $^{11}$C-raclopride displacement studies in striatum in man show an 11–13 h half-life. The example of pardoprunox also raises the issue of whether partial agonism as D2 receptors might be advantageous in preventing dyskinesia induction but the available data are equivocal and insufficient preclinical work has been undertaken.

What has emerged from CDS is that the delivery of dopaminergic drugs influences the onset of dyskinesia. Two broad approaches to this issue have evolved showing that it applies equally to L-DOPA and dopamine agonists. A series of studies in MPTP-treated primates showed that the continuous delivery of D2/D3 selective dopamine agonists by continuous subcutaneous infusion results in even lower levels of dyskinesia than seen on repeated oral administration or subcutaneous injection (Bibbiani *et al.*, 2005b; Stockwell *et al.*, 2008; Stockwell *et al.*, 2009). For example, continuous 24 h subcutaneous infusion of rotigotine using osmotic minipumps was used to achieve steady-state plasma levels and mimic delivery from a transdermal patch in man. Continuous delivery was associated with less dyskinesia than that produced by intermittent rotigotine administration or L-DOPA. Similarly, while repeated subcutaneous injection of the short-acting D1/ D2 dopamine receptor agonist apomorphine resulted in dyskinesia induction, continuous delivery from polymer rods impregnated with the drug and implanted subdermally resulted in minimal involuntary movements. Indeed, the most convincing demonstration of the potential of CDD in PD for avoiding dyskinesia induction has come from this approach and has led to the introduction of transdermal approaches to treatment and also to extended release once daily formulations of ropinirole and pramipexole.

Whether the same principle applies rigorously to the delivery of L-DOPA has still to be demonstrated. Intuitively, a formulation of L-DOPA that delivers dopamine to the striatum will remove pulsatile stimulation of postsynaptic dopamine receptors and prevent dyskinesia induction but there is little available evidence. L-DOPA has proved extremely difficult to produce in an extended or sustained release form. Many of the problems arise from the effect of erratic gastric emptying in PD on its absorption and the fact that L-DOPA is only absorbed by active transport from the upper small intestine. Immediate release L-DOPA was

compared to Sinemet CR for the prevalence of dyskinesia in early PD but over 5 years no difference was observed (Block *et al.*, 1997). Whether this reflects a lack of effect of continuous delivery, the minimal difference of half-life between the two forms or the generally low incidence of dyskinesia in early PD is not clear. More recently, the pharmacokinetic profile of L-DOPA plus a peripheral decarboxylase inhibitor has been improved by combination with a peripherally acting inhibitor of COMT, namely entacapone. Inhibition of peripheral COMT will prevent the peripheral breakdown of L-DOPA, and will provide a more sustained delivery of L-DOPA to the brain. Studies in the MPTP-treated primate suggest that this approach might lead to a reduction in dyskinesia induction (Smith *et al.*, 2005). Entacapone significantly increases the duration of antiparkinsonian action of L-DOPA in MPTP-lesioned primates. When administered four times daily with L-DOPA plus carbidopa, entacapone treatment resulted in less dyskinesia than treatment with L-DOPA plus carbidopa alone. However, to date translation of these effects to the clinic has not been successful. A clinical study in early PD, STRIDE-PD compared L-DOPA/carbidopa/entacapone (Stalevo) treatment with L-DOPA/carbidopa administered four times daily at 3.5 h intervals over 2 years with time to onset of dyskinesia as the primary endpoint (Stocchi *et al.*, 2010). Stalevo did not reduce the prevalence of dyskinesia with time but the dosing regimen used appears not to have produced CDD in man. However, it may be that this approach in general may not reduce dyskinesia induction by L-DOPA as continuous duodenal infusion of L-DOPA plus benserazide in 6-OHDA-lesioned rats failed to reduce the onset of the rodent equivalent of dyskinesia or AIMs compared to pulsatile L-DOPA treatment (unpublished data). Again this returns to the point that the difference between L-DOPA and dopamine agonists with respect to dyskinesia may be pharmacological in nature. Perhaps a glimmer of hope comes from the finding that reinstatement of dopamine production in the denervated caudate-putamen of MPTP-treated primates using a viral vector containing the genes for TH, AADC, and GTP cyclohydrolase (Prosavin) showed improved motor function in the absence of dyskinesia induction (Jarraya *et al.*, 2009). However, transplantation of foetal dopaminergic neurones into the putamen in PD has been associated with the induction of dyskinesia so making the overall effect of dopamine replacement difficult to assess (see Lane; this volume).

### C. Lessons from Trying to Control Established Dyskinesia through Dopaminergic Routes

Once dyskinesia has developed as a response to L-DOPA treatment, it is difficult to control the involuntary movements while needing to improve the motor symptoms of PD. All currently used dopaminergic medications from L-DOPA, dopamine agonists, COMT inhibitors, and MAO-B inhibitors add to the overall

dopaminergic load and provoke the same involuntary movements. The normal pharmacological strategies that relate to oral drug treatment involve cutting back on drug dosage or fractionating doses to avoid peak dose LID. But this is more witch doctoring than neurology and requires detailed attention to an individual patients treatment regimen and drug response. There is evidence, however, that CDD might be usefully employed. Indeed, the original definition of CDS related to the continuous infusion of L-DOPA, apomorphine, and lisuride in mid- to late-stage PD rather than to longer acting oral medications. Importantly, the continuous subcutaneous or intravenous infusion of apomorphine improves "on" time in patients with marked motor fluctuations and over time leads to a significant reduction in the intensity of dyskinesia that was present on oral medication (Katzenschlager *et al.*, 2005; Manson *et al.*, 2001). More recently, the continuous intraduodenal infusion of L-DOPA or L-DOPA methyl ester has been shown to have exactly the same effects with marked improvements in motor function being associated with significant reductions in LID (Antonini *et al.*, 2010; Karlsborg *et al.*, 2010; Nyholm, 2006; Stocchi *et al.*, 2005). Again there is a time factor with the reduction in dyskinesia occurring over a period of months. There does not seem to have been any experience of delivering other dopamine agonists in a continuous manner associated with decreased dyskinesia. It does not seem to be known whether a selective D2/D3 agonist would have the same effects. Why the decrease occurs is not well understood but must involve a resetting of basal ganglia output that requires continuous receptor stimulation and time for adaptive change to occur. It is also unclear whether the decrease in dyskinesia represents decreased expression of involuntary movements or whether de-priming has occurred. The long time course of the effect seems to suggest the latter but this does not seem to have been tested experimentally or in the clinic.

Other potential approaches to dealing with dyskinesia in the face of the need for continuing dopaminergic medication are either based on preclinical data or hypothetical in nature and have not been tested in man. The advantages of targeting D3 dopamine receptors with antagonists, such as S33084, have already been described. D2 agonists seem to express less dyskinesia than L-DOPA (see above) but it is not general clinical practice to switch to agonist therapy in later PD although there is some evidence that a switch to high doses of the long-acting dopamine agonist pergolide can reduce dyskinesia intensity (Facca and Sanchez-Ramos, 1996). This would be consistent with the finding that LID in MPTP-treated primates can be reversed over time by switching to the long-acting oral agonist cabergoline (Hadj *et al.*, 2000) or rotigotine (Stockwell *et al.*, 2010). Adding a D2 agonist to L-DOPA treatment is seldom used as a strategy to control LID in PD but in primates a combination of L-DOPA and pramipexole allowed a reduction in L-DOPA dosage, maintained or improved efficacy and reduced dyskinesia intensity (Tayarani-Binazir *et al.*, 2010). D2 partial agonists, such as pardoprunox, might also offer an advantage when administered together with L-DOPA as in

MPTP-treated primates, the combination increased antiparkinsonian efficacy but decreased dyskinesia intensity (Tayarani-Binazir *et al.*, 2010). The D1 receptor might perhaps surprisingly offer advantages for established dyskinesia. In MPTP-treated primates with LID, a comparison of equivalent doses of L-DOPA, D1 and D2 agonists showed less expression of LID with selective D1 agonists but no difference between L-DOPA and D2 agonists (Blanchet *et al.*, 1993). In PD, too little has been done to determine the translational value of this finding. The only real experience is with ABT-431 that was shown to provoke dyskinesia in PD with LID but the intensity of involuntary movements relative to L-DOPA or efficacy was not assessed (Rascol *et al.*, 1999; Rascol *et al.*, 2001). Lastly, and perhaps most speculatively, the MPTP-treated primate suggests that monoamine reuptake blockers, such as brasofensine, possess antiparkinsonian effects that are not associated with the expression of established dyskinesia (Hansard *et al.*, 2002; Hansard *et al.*, 2004; Pearce *et al.*, 2002). The problem with these findings is that the efficacy was not reproduced in early clinical trials in PD and this remains as a failure of the predictive value of the primate model (Bara-Jimenez *et al.*, 2004).

## IV. Nondopaminergic Approaches to LID

The complexity of the basal ganglia offers the opportunity for modulating striatal output through multiple neurotransmitter systems and this, in turn, provides numerous pharmacological targets that might be able to prevent the onset of dyskinesia or to attenuate the intensity of established involuntary movements. In this part of the chapter, we will consider some of these pathways and look at the evidence that their modulation might be functionally useful in the clinical control of dyskinesia in PD.

The mechanisms underlying the expression of LID, once this has developed, have received significant attention over the last decade and, in contrast to those responsible for priming, are better understood (see other chapters in this volume) (Bezard *et al.*, 2001; Cenci and Lindgren, 2007; Jenner, 2008). There is an appreciation of changes occurring beyond dopamine receptors that alter intracellular signaling cascades and synaptic plasticity and these relate to imbalances in the activity of the direct and indirect striatal output pathways. A core component of the mechanisms underlying LID expression may be an overactivity of the D1-mediated direct pathway resulting in an inability to alterations in a synaptic process responsible for long-term potentiation of synaptic efficacy or alterations in the intracellular signaling cascades downstream of the G proteins to which D1 receptors couple (Bezard *et al.*, 2005). However, LID is not a single entity and ideas have begun to emerge as to how LID of a choreic nature might differ

mechanistically from that of dystonic LID. In addition, the expression of LID may be focal, segmental, or generalized and this may relate to discrete alterations in firing patterns in the target of the direct pathway, namely the internal segment of the globus pallidus (GPi). Underactivity in the indirect pathway may also contribute to LID but this remains disputed. The concept is broadly supported by a large body of literature, and alterations in the indirect pathway might play a role in defining the phenomenology of LID (Bezard *et al.*, 2001).

The importance of this discussion resides in the fact that both the direct and indirect pathways are primarily composed of GABAergic neurones but are exceptionally rich in neuromodulators that control motor function. These give a wide range of drug targets that could, theoretically, modulate firing of indirect and direct pathway neurones and thus modify LID. There is a significant opportunity to develop an approach that is focused on nondopaminergic agents and employs them as adjuncts to the dopaminergic treatment of PD to modify the activity of the direct and indirect pathways so as to prevent the onset of dyskinesia or to its suppression once established. The challenge is to define approaches that can achieve the desired modulation of basal ganglia output, whether it be of the direct or indirect pathway. Given the critical involvement of these pathways in motor function, a major challenge is to identify drugs that have a wide therapeutic window which allows suppression of dyskinesia without inhibition of antiparkinsonian benefit. Similarly, there has to be a selectivity of action between the effects exerted on the direct and indirect pathways to ensure that the beneficial effect on dyskinesia is not cancelled out or that chorea is converted to dystonia or vice versa as can occur through manipulation of cholinergic function.

### A. The Experience with Glutamate Antagonists

The glutamatergic manipulation of basal ganglia output forms a strong candidate for the drug treatment of LID. The cortico-striate pathway has been shown to be altered following AIMs induction in 6-OHDA-lesioned rats with alterations in LTD and abnormal storage of information (Cenci and Konradi, 2010; Kobylecki *et al.*, 2010). Since it activates glutamatergic receptors on medium spiny neurones making up the direct and indirect pathways, this may be a key target. When this is coupled with the over-activity of the subthalamic nucleus in PD/LID, then a clear rationale for the use of glutamate antagonists is established. Indeed, the only compound approved for the treatment of dyskinesia in PD is the weak NMDA antagonist, amantadine which can provide long-term suppression of involuntary movements. However, amantadine is not well-tolerated by many patients and it can be difficult to reach effective doses. In addition, amantadine is nonspecific in its activity and it has significant dopaminergic properties and there are also reports of anticholinergic activity. For these reasons, alternative molecules

had to be sought to determine the effectiveness of NMDA antagonists in suppressing established dyskinesia. The rationale for this approach was validated by subsequent studies that showed abnormal expression, and phosphorylation of striatal NMDA receptors occurs in both rodent and nonhuman primates models of LID (Dunah *et al.*, 2000; Hallett *et al.*, 2005, 2006). This along with evidence of abnormalities in synaptic plasticity typically associated with NMDA signaling, provided cellular and molecular mechanisms by which the direct pathway might become overactive in LID (Cenci and Konradi, 2010).

As adjunctive therapies to suppress the expression of established dyskinesia, NMDA receptor antagonists have been very successful. Indeed, in both rodent and primate models of PD, amantadine and other NMDA antagonists generally show good antidyskinetic benefit, without compromising the antiparkinsonian effects of L-DOPA. The most effective have been those antagonists that are selective for the NR2B receptor. Perhaps importantly, MPTP treated primates, while reducing dyskinesia overall, NMDA antagonists are most effective against chorea. This suggests that some glutamatergic therapies for suppressing established dyskinesia may have more benefit in patients whose LID is chorea or dystonia dominant or *vice versa*. In addition, NMDA antagonists may also exacerbate dyskinesia and there are clinical reports of marked worsening of dyskinesia after withdrawal of amantadine, perhaps suggesting an upregulation of receptor function following blockade. To date, no NMDA antagonist with the exception of amantadine has been successfully developed for the treatment of dyskinesia in PD. This may relate to the difficulty in identifying a subtype of the NMDA receptor that is localized to basal ganglia and will not induce marked side effects through actions on NMDA receptors in other brain regions.

As a consequence, the focus of research has been broadened to other classes of glutamatergic receptor and to the AMPA receptor (Bibbiani *et al.*, 2005; Kobylecki *et al.*, 2010; Perier *et al.*, 2002). Within the striatum of rodent and primate models of LID, AMPA receptors are abnormally phosphorylated and appear to have increased targeting to the synapse (Silverdale *et al.*, 2010). These changes may underpin enhanced AMPA-mediated excitation of the direct pathway in LID. Indeed, AMPA antagonists, including talampanel and topiramate, can suppress AIMs and dyskinesia in the 6-OHDA rodent and MPTP-treated primate without compromising the antiparkinsonian activity of L-DOPA (Silverdale *et al.*, 2005). Both talampanel and topiramate were advanced to proof-of-concept clinical studies in patients with established LID. While topiramate was found to be poorly tolerated and the study discontinued, phase III studies to assess the ability of talampanel to suppress established dyskinesia were completed but the findings have never been made public. So far attacking ionotropic glutamate receptors for the treatment of LID in PD has not been a therapeutic success.

A third approach to reducing excitatory transmission and suppressing LID has been that focused on metabotropic glutamate receptors and specifically,

antagonists of mGluR5. mGluR5 is expressed in relatively high levels throughout the basal ganglia and its levels are increased in the MPTP-lesioned primate (Samadi *et al.*, 2008). In 6-OHDA-lesioned rodents, the mGluR5 antagonists, MTEP and MPEP, can reduce AIMS. In nonhuman primates, mGluR5 antagonists, including MTEP, AFQ056, and fenobam, are efficacious in reducing L-DOPA-induced dyskinesia (Gregoire *et al.*, 2011; Johnston *et al.*, 2010; Morin *et al.*, 2010). Of these, AFQ056 has advanced to clinical evaluation, where at Phase II proof-of-concept it displayed modest antidyskinetic efficacy without impairing the antiparkinsonian efficacy of L-DOPA (Berg *et al.*, 2011). In contrast to this and to some studies in MPTP-treated primates, a single primate study with MTEP did suggest a modest loss of antiparkinsonian benefit with the doses of MTEP that were most effective in suppressing dyskinesia (Johnston *et al.*, 2005). This latter finding raises caution as to whether the therapeutic window for mGluR5 antagonists will be wide enough to be used with ease in clinical practice.

The question of whether the initial introduction of a glutamate antagonist into therapy in PD would prevent the initiation of dyskinesia in response to L-DOPA therapy is important. Indeed, NMDA, AMPA, and metabotropic glutamate receptor antagonists have all shown an ability to prevent priming and stop the development of AIMs in 6-OHDA-lesioned rats. In the nonhuman primate, the only compound that has been assessed is the NR2B selective NMDA antagonist besondopril. In the MPTP-lesioned primate, initiation of besondopril treatment with L-DOPA significantly reduced the rate of development of LID which was essentially absent. The difficulty with the interpretation of this study relates to whether priming for dyskinesia was prevented or whether the effects observed reflected the ability of NMDA antagonists to suppress the expression of LID. Unfortunately, the animals were not challenged with L-DOPA at the end of the study in the absence of besondopril to solve this dilemma. Data on clinical studies to evaluate the effects of besondopril in PD have not been reported to date. It is really surprising that more is not known in primates concerning the effects of glutamate antagonists on the development of LID. What is really surprising is that the ability of amantadine to prevent dyskinesia induction has not been studied in MPTP-treated primates or in patients with PD despite its quite common use in treatment of early motor symptoms.

### B. Targeting A2a Receptors on the Indirect Pathway

The A2a adenosine receptor is relatively enriched on medium spiny neurones of the indirect pathway compared to other neuronal populations (Morelli *et al.*, 2007; Schwarzschild *et al.*, 2006). The localization on the cell bodies in the striatum and on the terminals of this GABAergic pathway in the GPe provides a selective means of manipulating basal ganglia output. In addition, the adenosine A2a

receptor can modulate dopaminergic, cholinergic, and glutamatergic function in the striatum. It provides an attractive target for a systemically administered approach to reducing the activity of the indirect pathway in PD so improving motor function without the development of dyskinesia. The reality is that A2a antagonists by themselves do not induce marked rotation in 6-OHDA-lesioned rats or markedly improve motor function in MPTP-treated primates. There are no reports of improvements in motor symptoms of PD when A2a antagonists are used as monotherapy. Rather there is a synergism with dopaminergic drugs and improvements in motor function are seen in rodents, primates, and man when utilized in this manner. It was initially suggested that A2a antagonists would improve motor function without worsening established dyskinesia but this was based on a misreading of the literature. In fact, it had been shown that the A2a antagonist istradefylline did not itself provoke dyskinesia in L-DOPA primed MPTP-treated primates but the effects of combinations with L-DOPA were not studied for their effects on LID (Kanda *et al.*, 1998). In clinical study, istradephylline improved "on" time in PD but at the expense of a worsening in nontroublesome dyskinesia. This may be a general effect of this class of drug although similar findings were not made in phase II studies with another A2a antagonist preladenant.

So, selective effects on the indirect output pathway do not seem to provoke a robust antiparkinsonian effect but does not provoke established LID. So the key question becomes whether A2a antagonists can prevent the onset of dyskinesia and here, at least, there are some positive findings. In rodents, administration of A2a antagonists to previously drug naive 6-OHDA-lesioned rats does suppress the development of L-DOPA-induced abnormal involuntary movements (AIMs). Likewise in mice where the gene encoding the A2a adenosine receptor has been knocked down, the ability of repeated L-DOPA treatment to induce AIMs is much reduced. In the MPTP-lesioned primates, the A2a antagonist istradefylline essentially abolished the ability of the short-acting D1/ D2 dopamine receptor agonist apomorphine, to induce dyskinesia without reducing the improvement in motor function produced by apomorphine (Bibbiani *et al.*, 2003). This effect appeared to be a true prevention of priming for dyskinesia since on withdrawal of istradephylline, continuation of apomorphine treatment alone did not initially result in dyskinesia but then involuntary movements started to appear at the same rate as in drug-naive animals treated with apomorphine alone. A fly in the ointment is the results of an unpublished study of the effects of istradefylline administered in combination with L-DOPA to previously drug-naive MPTP-treated primates. The administration of istradefylline enhanced the improvement in motor disability produced by L-DOPA in line with expectations. However, it did not prevent or slow dyskinesia induction produced by L-DOPA treatment and even enhanced peak effects in the early stages of treatment in line with clinical findings on the use of istradefylline in PD. So, it may be that the role of A2a antagonists in PD may be

in allowing the use of L-DOPA sparing strategies that allow a dose reduction while maintaining the improvement in motor function seen at higher doses and with minimal worsening of LID. What is needed now is a greater experience of the use of A2a antagonists in man and the initiation of trials in early PD with end points related to time to dyskinesia appearance.

### C. 5-HT Neurones and Receptors and the Inhibition of LID

There is an intimate relationship between dopaminergic and serotoninergic transmission in brain in the control of motor function and this is disturbed in PD. While neuronal loss occurs in the raphe nuclei leading to a fall in forebrain serotonin content, a different series of events appear to occur in the striatum. The loss of nigrostriatal neurones leads to a hyperinnervation by serotoninergic fibers that can be seen in 6-OHDA-lesioned rats, MPTP-treated primates, and in PD. This may in itself have functional consequences as 5-HT receptors ($5\text{-HT}_{1A}$ and $5\text{-HT}_{2A}$) are present on medium spiny output neurones and on the terminals of glutamatergic fibers of the corticostriatal tract. By regulating striatal output and glutamate release, drugs acting through serotoninergic mechanisms may be able to manipulate motor function in PD although no agent has been brought through clinical evaluation for symptomatic treatment. However, in LID there are clear morphological abnormalities in serotoninergic fibers in the striatum seen in AIMs in rats, dyskinesia in MPTP-treated primates, and in dyskinetic individuals with PD (Rylander *et al.*, 2010). The importance of these changes is not entirely clear but they seem to be associated with the uptake of L-DOPA into serotoninergic terminals, its conversion to dopamine by AADC and its nonphysiological release on to dopamine receptors (Carta *et al.*, 2007). This may have a crucial role to play in LID since destruction of the serotoninergic system in 6-OHDA-lesioned rats prevents the onset of AIMs on subsequent L-DOPA treatment (Munoz *et al.*, 2009). This provides a potential therapeutic target for LID as the synthesis and nonphysiological release of dopamine from serotoninergic neurones is regulated by $5\text{-HT}_{1A}$, $5\text{-HT}_{1B/D}$, and $5\text{-HT}_{2A}$ receptors. Notably, $5\text{-HT}_{1A}$ agonists have been shown to suppress dyskinesia in 6-OHDA-lesioned rats exhibiting AIMs and in MPTP-treated primates with LID but with disagreement over whether this occurred without impairing the beneficial motor effects of L-DOPA (Gerlach *et al.*, 2011, 2009; Iravani *et al.*, 2006; Munoz *et al.*, 2008). In Phase II studies in PD, the $5\text{-HT}_{1A}$ agonist sarizotan reproduced the effects on dyskinesia although a worsening of motor symptoms occurred but was ascribed to disease progression over the course of the study (Goetz *et al.*, 2007; Olanow *et al.*, 2004). However, in Phase III evaluation, in two independent studies, PADDYI and PADDYII, sarizotan did not alter the antiparkinsonian effects of L-DOPA but it was no more effective than placebo in reducing dyskinesia. In subsequent studies in MPTP-treated primates, the issue seems to have been resolved

by careful dose titration with sarizotan (Gregoire *et al.*, 2009). The therapeutic window for antidyskinetic efficacy appears to be narrow, with inhibition of the antiparkinsonian activity of L-DOPA occurring at higher doses so making clinical development difficult. Subsequent preclinical investigations have shown that a combination of 5-$HT_{1A}$ and 5-$HT_{1B}$ agonist activity may produce a more robust inhibition of AIMs in 6-OHDA-lesioned rodents and of dyskinesia in MPTP-treated primates (Munoz *et al.*, 2008) and that 5-$HT_{2A}$ antagonists (Oh *et al.*, 2002) have utility in reducing dyskinesia. Indeed, the 5-$HT_{1A/1B}$ agonist, eltoprazine is currently undergoing clinical evaluation for the treatment of LID in PD. In further clinical studies, two other serotonergic agents, quetiapine and clozapine, have been successful in reducing established dyskinesia in both MPTP-treated primates (Oh *et al.*, 2002; Visanji *et al.*, 2006) and Phase II studies in PD though those of quetiapine were not confirmed with subsequent Phase IIb studies (Katzenschlager *et al.*, 2004). The effects seen have been ascribed to a 5-$HT_{2A}$ antagonist like action, though it is probably fair to term these compounds as having mixed serotoninergic activity. Buspirone has recently been demonstrated to suppress dyskinesia in patients with PD who had received a human fetal nigral cell transplant some years ago and were now exhibiting involuntary movements in the "off" state (Politis *et al.*, 2010). A more selective 5-$HT_{2A}$ ligand, a partial agonist ACP-103, is currently being evaluated for antidyskinetic actions in PD and may better address the value of this target.

### D. Noradrenergic Receptors and Attempts to Modify LID

In a similar manner to serotonin, there is a well-established relationship between noradrenergic and dopaminergic transmission in the control of motor function. Noradrenergic cells in the locus coeruleus degenerate in PD leading to a fall in forebrain noradrenaline content. This is partially replenished by L-DOPA as some of the dopamine formed is subsequently converted to noradrenaline. This might in part explain why L-DOPA possesses greater efficacy and has a greater liability to induce dyskinesia, than occurs with dopamine agonist drugs. Noradrenergic receptors (α-2a,b and c) are present on medium spiny neurones in the striatum so forming a target for therapeutic intervention. However, there is little evidence of innervation by noradrenergic fibers, which raises the question of their physiological role. One suggestion is that they may in reality act as low-affinity dopamine receptors that play a regulatory role to modulate dopaminergic transmission in conjunction with the high-affinity D1- and D2-like receptors on output neurones. However, it is feasible that their modulation might form the basis of an antidyskinetic therapy and this has been explored in both animals and man. The α-2 antagonist idazoxan was shown to decrease AIMs in 6-OHDA-lesioned rats and LID in MPTP-lesioned primates without impairing the efficacy of L-DOPA and it suppressed dyskinesia in Phase II evaluation in PD (Buck *et al.*, 2010;

Grondin *et al.*, 2000; Rascol *et al.*, 2001). However, a large Phase III study failed to identify an antidyskinetic action of the drug and the program was terminated. The α-2 antagonist fipamezole was also shown to be similarly effective in rat and rodent models of LID (although the findings were not universally positive) and it was able to suppress dyskinesia (Savola *et al.*, 2003) and to enhance the duration of action of L-DOPA in a small group of patients with PD receiving acute intravenous L-DOPA infusions. However, the drug was not well absorbed orally and a novel buccal spray formulation was devised for subsequent evaluation. A recent Phase IIb study failed to confirm the antidyskinetic actions of fipamezole when the whole treatment population was analyzed but there was a positive effect when only those patients in North American centers were included and compliance issues may have tainted the overall trial outcome. However, at this point in time, no other noradrenergic approaches are being pursued for the suppression of LID in PD.

### E. Other Approaches

Basic research on neurotransmission within the basal ganglia continues to highlight additional mechanisms by which adjuncts to L-DOPA might be developed and suppress established LID. Of these, several have shown efficacy in MPTP-treated primates and are being, or have potential to be translated to the clinic. These include mu opioid antagonists, histamine H2 receptor antagonists, histamine H3 receptor agonists, and CB1 receptor agonists and antagonists. The list of agents with demonstrated efficacy in reducing AIMs in 6-OHDA-lesioned rats includes some interesting and very druggable targets, for example, fatty acid amide hydrolase (FAAH) inhibitors and PPAR-α. However, their effectiveness in MPTP-treated primates and in man remains to be investigated.

### F. Other Therapies Aimed at Reversing Priming for LID

The process of priming of basal ganglia for the induction and subsequent expression of LID is poorly understood. Notably, the persistence, if not permanent nature of the involuntary movements suggests a phenomenon that must involve changes in motor programs laid down in basal ganglia for the execution of voluntary movement and suggests a key role for processes involving LTP/LTD or the like (Calabresi *et al.*, 2008; Picconi *et al.*, 2003). The persistence is remarkable since in MPTP-treated primates, once dyskinesia is established by even short periods of L-DOPA treatment, the same involuntary movements can be provoked by single L-DOPA challenges even if the animal has remained drug free for many months in the interim. This probably explains the difficulties experienced in reversing priming and the limited measures that can be taken once dyskinesia has become established (see above).

There are three potential approaches to the problem, some of which will be dealt with elsewhere in this volume but are worthy of mention at this point. The majority are currently theoretical and involve the restoration of normal basal ganglia function through cellular or viral vector technologies. First, it may be possible to undo the changes that lead to the establishment of an abnormal basal ganglia. For example, the administration of the gene for GAD in to the subthalamic nucleus is reported to have a positive effect on motor symptoms of PD (in a similar manner to DBS) and the same approach could be conceived as being able to reverse dyskinesia through an increase in GABAergic tone (Kaplitt *et al.*, 2007; LeWitt *et al.*, 2011). Second, the restoration of nigrostriatal function could undo the effect that denervation has in gating the sensitivity of the basal ganglia to L-DOPA and dyskinesia. For example, dopamine production in the striatum through the implantation of a vector containing the genes for TH, AADC, and GTP-cyclohydrolase (ProSavin) restores motor function but without dyskinesia induction and again, could be seen as having positive effects on established dyskinesia (Jarraya *et al.*, 2009). Recently, a viral vecior delivering the genes for TH and GTP-cyclohydrolase was shown to prevent priming for AIMs in 6-OHDA-lesioned rats suggesting a way forward that might also reverse involuntary movements (Bjorklund *et al.*, 2010). This, however, should be balanced against the induction of dyskinesia in the "off" state produced by implantation of fetal dopaminergic cells (see Lane *et al.*, this volume). Restoring nigral TH positive cell numbers may potentially "deprime" the basal ganglia. In studies involving the direct intracerebral injection of the trophic factor, GDNF in MPTP-treated primates with established LID, a small improvement in the number of TH positive cells in substantia nigra had a significant effect on the intensity of dyskinesia produced by L-DOPA challenge (Iravani *et al.*, 2001). Cell- and gene-based approaches are now being developed to look at the effects of GDNF on motor components of PD. A related approach involves the use of a viral vector delivery the gene for neurturin, another member of the GDNF super family (Gasmi *et al.*, 2007; Grondin *et al.*, 2008; Herzog *et al.*, 2009; Marks *et al.*, 2010). This improves motor function in MPTP-treated primates through an action leading to increased TH positive cell numbers in substantia nigra. Once more, this may be advantageous in reversing the process underlying dyskinesia in PD but it has yet to be investigated and recent clinical studies failed to reproduce the effects of neurturin seen in primates. Finally, it may be possible to interfere at the molecular level in signaling pathways that are critical to the maintenance of priming for dyskinesia. For example, nitric oxide is crucial as a signaling pathway for the induction of LTP/LTD and nitric oxide synthase inhibitors appear to have effects at least on established dyskinesia. Their effects on dyskinesia induction remain unknown. The Ras-Erk pathway has been linked to LID induction and inhibition of Ras-guanine nucleotide-releasing factor 1 (RAs-GRF1) signaling in the striatum reverses motor symptoms associated with L-DOPA induced AIMs in 6-OHDA-lesioned rats (Fasano *et al.*, 2010).

## V. Conclusions

In conclusion, there is no shortage of targets or classes of compound with potential to suppress established LID. Several classes show efficacy in nonhuman primate models and these models appear to predict efficacy in first in man, Phase IIa proof-of-concept studies. However, failures at Phase IIb and Phase III are a recurring theme with dyskinesia studies. The only widely available drug to suppress dyskinesia, amantadine was able to avoid this Phase III process as it was already marketed for PD and has proved a valuable approach in a significant proportion of patients with LID. A major challenge in reducing the burden of LID falls on understanding how to surmount the Phase III hurdle.

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# SURGICAL APPROACH TO L-DOPA-INDUCED DYSKINESIAS

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Many patients treated chronically with L-dopa for Parkinson disease (PD) become functionally disabled by L-dopa-induced dyskinesias (LID). Evolved from early empirical procedures, modern stereotactic surgical lesioning techniques and deep brain stimulation (DBS) can effectively treat LID while simultaneously improving the cardinal motor symptoms of PD. Here we review the common surgical targets used to treat LID, and compare their relative efficacy. We explain the anti-dyskinetic action of surgery at each of these targets based on evolving models of basal ganglia function. Finally, we discuss the appropriate selection of patients with LID for surgery and address relevant technical and management issues in these patients.

## I. Introduction

L-dopa was first introduced for the treatment of Parkinson disease (PD) in the 1960s by Cotzias *et al.* (1969), and brought with it a profound beneficial effect on the cardinal motor symptoms of the disease. Unfortunately, within a decade, the unquestioned efficacy of L-dopa was partially offset by the appearance of several limitations. L-dopa did not prevent the progression of PD and its chronic use was

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00006-7




0074-7742/10 $35.00

often associated with a predictable overall loss of drug effect throughout the course of the day ("wearing off"), less predictable fluctuations in efficacy ("on–off" fluctuations), and involuntary movements now recognized as L-dopa-induced dyskinesia (LID). These complications are observed in as many as 50%—with frank dyskinetic activity seen in upwards of 80%—of patients treated with L-dopa for more than 5 years (Rascol, 2000; Rascol *et al.*, 2000).

For some patients, LID may be as disabling as the underlying symptoms of PD, and a reduction in LID can produce an overall improvement in motor function, the ability to perform activities of daily living, and self-esteem (Follett, 2004). Typically, LID is managed by adjustments in the dose and timing of administration of L-dopa. In the setting of severe dyskinesia this means that there may be a need to reduce the dose of L-dopa altogether with an associated, undesirable loss of control of the cardinal symptoms of PD. Consequently, patients are often faced with the unpleasant choice between accepting more dyskinesias with better control of PD or settling for dyskinesias which are less severe but accompanied by a worsening of off period PD symptoms. In such patients, stereotactic surgery of the basal ganglia, either by lesioning or high frequency deep brain stimulation (DBS), can be effective. An understanding of the various surgical options to treat LID is particularly timely, given the increasingly established role of DBS for moderate to advanced PD.

In this review, we summarize approaches to the surgical management of LID in patients with PD. Beginning with an overview of the current definition of LID in the neurosurgical literature we address some of the mechanistic and physiological underpinnings of LID. We then summarize the effectiveness and proposed mechanisms of action of surgery for LID in the three brain structures most commonly targeted in PD: the thalamus, the globus pallidus interna (GPi), and the subthalamic nucleus (STN). Finally, we describe a modern neurosurgical approach to the treatment of LID including patient selection, choice of target, and a brief consideration of technical issues including DBS programming.

## II. Brief Overview of LID

### A. Clinical Definition

LID occurs after the prolonged chronic daily use of L-dopa, though the duration between the initiation of L-dopa therapy and the development of LID is variable. The clinical expression of LID is a complex series of involuntary movements that has been divided into three distinct patterns (Guridi *et al.*, 2008; Obeso *et al.*, 2000). Peak dose dyskinesias occur during the "on" medication state and are associated with a high serum concentration of L-dopa and, typically, maximal anti-

parkinsonian effect. Biphasic dyskinesias are characterized by repetitive stereotyped movements occurring just before or just after the period of maximal L-dopa benefit. Frequently, biphasic dyskinesias are worst in the lower extremities. Finally, "off" period dystonia occurs in conjunction with the lowest plasma levels of L-dopa and is characterized by sustained, painful postures primarily of the feet. Recent reports have suggested that varying genetic susceptibilities may pre-dispose PD patients to one subtype of LID over another (Lee *et al.*, 2011). Ideally, surgical intervention alleviates all three forms of LID, though many published reports in the neurosurgical literature do not distinguish between these subtypes.

### B. Causative Mechanisms Underlying LID and Implications for Surgical Treatment

The precise pathophysiological mechanisms underlying LID are still being elucidated and are addressed in detail elsewhere in this book. In brief, data from classical stereotactic lesioning procedures in the pre L-dopa era, intra-operative microelectrode recordings (MERs) during modern DBS surgery, and from animal models of Parkinsonism—chiefly the MPTP (1-methyl-4-phenyl-1-2-3-6-tetrahydropyridine) model in monkeys—have provided reasonable explanations for the efficacy of certain surgical targets in the treatment of LID, while simultaneously creating paradoxes in our understanding of basal ganglia function and the changes in basal ganglia physiology induced by surgery.

In the pre L-dopa era, there was no effective medical treatment for so-called "hyperkinetic" conditions such as chorea or hemiballism (Guridi *et al.*, 2008), which share many common features with LIDs. This stimulated an intense search for surgical treatment targets, and motivated several studies examining the effects of lesions in the basal ganglia and motor thalamus of experimental animals. In 1949, Whittier and Mettler (1949) showed that lesioning the STN in rhesus monkeys produced severe contralateral hemiballism, which was only expressed in the presence of an intact pallidum and ansa lenticularis. With Carpenter *et al.* (1950), they went on to show that hemiballism produced by an STN lesion was partially reversed by lesioning the pallidum or its outflow tract to the pallidal receiving portion of the thalamus. Subsequently, Talairach *et al.* (1950) were able to produce sustained improvement in a human patient suffering from hemiballism by partial coagulation of the Gpi and ansa lenticularis via an open surgical approach. Similar results were obtained by Cooper (1981). Later, Mundinger *et al.* (1970) showed that lesions in the zona incerta or in the ventrolateral pallidal-receiving portions of the thalamus were effective against medically refractory extrapyramidal dyskinesias. In summary, these early studies established two key points: (1) that the pallidothalamic pathway is involved in the generation and/or maintenance of dyskinesias and (2) that lesioning the pallidum or

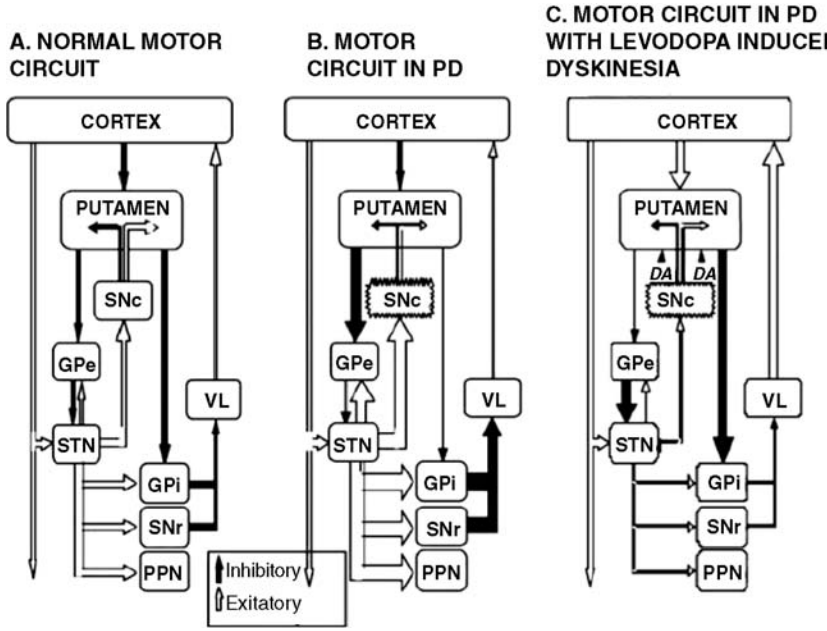


FIG. 1. The "rate" model of basal ganglia function in the setting of (A) normal motor function, (B) Parkinson Disease (PD), and (C) L-dopa-induced dyskinesia (LID). In PD, the net effect of reduced dopaminergic outflow from the substantia nigra pars compacta (SNc) is to increase the inhibitory effect of the globus pallidus interna (GPi) on the thalamus and reduce the thalamocortical drive in favor of movement. Conversely, pulsatile L-dopa administration causes dyskinesia by abnormally increasing thalamocortical drive. This occurs in two ways: (1) by reducing the inhibitory tone of striatal neurons in the indirect pathway, in turn increasing inhibition of the subthalamic nucleus (STN), and reducing the activation of the GPi and (2) by an increase in inhibition of the GPi directly by connections from the striatum. SNr = substantia nigra pars reticulata, VL = ventrolateral nuclei of the thalamus, PPN = pedunculopontine nucleus. With permission from Guridi *et al.* (2008).

interrupting the pallidothalamic pathway has an anti-dyskinetic effect. Accordingly, as we discuss later in this review, lesioning or stimulation within components of the pallidothalamic pathway have become established treatments of LID in the modern era of stereotactic functional neurosurgery.

In 1980s, the seminal "rate" model evolved to explain basal ganglia function and the cardinal features of PD (Alexander *et al.*, 1986, 1990) (Fig. 1). In PD, depletion of dopaminergic neurons in the substantia nigra pars compacta (SNpc) alters the output of the both the direct and indirect pathways to result in a net increase in the mean firing rate of the GPi, which in turn inhibits thalamocortical drive. By contrast, in LID the net effect of replenishing L-dopa should be a reduction in the mean firing rate of the GPi, in turn disinhibiting thalamocortical motor projections, producing an abnormal increase in cortical drive, and,

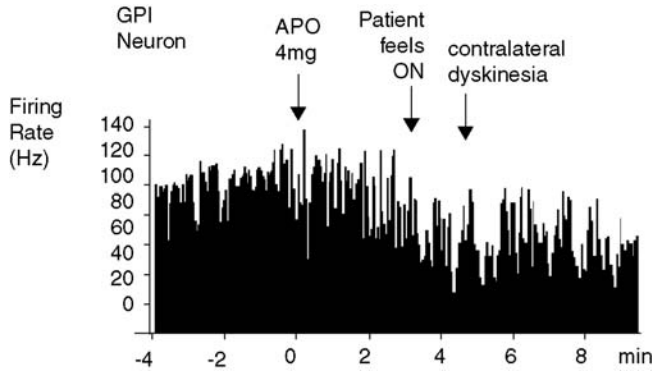


FIG. 2. Firing rate of a neuron in the globus pallidus interna (GPi) from a patient with L-dopa responsive Parkinson Disease (PD) demonstrating slowed firing accompanied by contralateral dyskinesia in response to apomorphine (a dopamine agonist). With permission from Hutchinson *et al.* (1997).

ultimately, resulting in abnormal or excessive movement (Albin, 1995; Guridi *et al.*, 2008). In MPTP-treated monkeys, L-dopa administration has indeed been associated with an inverse relationship between the mean firing rate of GPi neurons and the severity of dyskinesias (Papa *et al.*, 1999). Similarly, in humans, we have shown with intra-operative single-unit recordings that administering apomorphine—a dopamine agonist which, like L-dopa, causes dyskinesias in parkinsonsian patients—results in a significant reduction in the mean firing rate of neurons in both the GPi and STN during the peak on period (Hutchinson *et al.*, 1997; Levy *et al.*, 2001; Lozano *et al.*, 2000) (Fig. 2). In addition, metabolic imaging data have confirmed that there is a downstream increase in cortical activity during peak dose LID, particularly in the supplementary motor area (SMA). These changes are not observed in PD patients without LID (Rascol *et al.*, 1998).

Unfortunately, the rate model of basal ganglia function cannot fully explain the efficacy of surgical procedures originally applied empirically to treat both hypokinetic and dyskinetic conditions alike. Some have called this the Marsden and Obeso paradox (Marsden and Obeso, 1994). If PD and other hypokinetic states are the product of an overinhibited thalamus, then thalamotomy, which has long been used in treating PD, should worsen parkinsonian symptoms. Similarly, if dyskinesias are the exclusive consequence of a reduced firing rate in GPi neurons, then pallidotomy—which further reduces pallidal output—should actually worsen LID rather than improve it. To explain this paradox, several authors have invoked the existence of patterns of abnormal firing activity that accompany changes in firing rate in patients with LID (Krack *et al.*, 1998a, 1998b; Wu *et al.*, 2001). We and others have found supporting *in vivo* evidence of such patterns from intra-operative recordings (Lozano *et al.*, 2000; Obeso *et al.*, 2000). In particular, changes in the oscillatory activity of the basal ganglia seem to be important. As $\beta$-band (20 Hz)

activity appears associated with the parkinsonian state (Brown, 2003), so too is data now suggesting that LID is related to prominent low frequency activity in the 4–10 Hz range (Alonso-Frech *et al.*, 2006). The direct anti-dyskinetic effects of surgery, whether lesioning or DBS, may therefore be due to modifications in pathological oscillations that predominate in LID. Indeed it appears that "no news" from the pathological motor basal ganglia as occurs with lesions is better than "bad news" associated with either parkinsonism or dyskinesias. We will address the specific anti-dyskinetic mechanisms of surgery at each of the common surgical targets in the next section.

## III. Surgical Treatment of LID: Efficacy and Mechanisms of Action by Target

### A. Thalamus

#### 1. *Thalamotomy and Thalamic DBS*

In the pre L-dopa era, Hassler and Reichert (1954) performed the first thalamotomy in 1954, with the primary aim of treating tremor. Initially, they targeted the ventral oral nucleus complex which Hassler further subdivided into posterior (VOP) and anterior (VOA) subdivisions. This target proved somewhat ineffective for tremor control, and the target was eventually moved to Hassler's ventral intermediate nucleus (VIM) which corresponds to the cerebellar terminal territory of the thalamus, as opposed to the pallidal receiving portion. The VIM went on to become an established site for lesioning procedures (Ohye *et al.*, 1976) to control Parkinsonian and non-Parkinsonian tremor alike.

VIM thalamotomy has generally proven to be of little or limited effectiveness against LID (Guridi *et al.*, 2008). Some evidence for an anti-dyskinetic effect has been suggested by studies where a lesion in the VIM prevented the later development of LID (Cardoso *et al.*, 1995; Ohye *et al.*, 1976), but the exact location of the thalamic lesion in these reports—specifically whether they were consistently placed within VIM—is uncertain. Most studies have shown no effect altogether (Diederich *et al.*, 1992), and it may simply be that in patients with PD-associated tremor, successful VIM thalamotomy may permit a reduction in L-dopa dose with a subsequent indirect improvement of LID (Nagaseki *et al.*, 1986). To date the most convincing evidence arguing against the direct anti-dyskinetic effect of VIM thalamotomy comes from a study by Narabayashi *et al.* (1984). These authors examined patients who had undergone thalamotomy at either the VIM or VOA/VOP sites prior to being treated with L-dopa. They found that patients with lesions in the VOA/VOP region did not develop LID after L-dopa therapy was initiated, while those with VIM lesions did. This is in keeping with the presumed anti-

dyskinetic effect of lesions within the pallidothalamic pathway, and has been reproduced under controlled conditions in MPTP monkeys (Page *et al.*, 1993). Despite these findings, lesioning of the VOA/VOP has rarely been the procedure of choice among functional neurosurgeons and there are no robust long-term data on its sustained efficacy against LID.

High-frequency DBS of the VIM as first employed by Benabid *et al.* (1996) has an excellent effect against Parkinsonian tremor but has had little success in abolishing LID. Similarly, two of the largest VIM DBS series found either an insignificant decrease or no change altogether in LID (Limousin *et al.*, 1999; Tasker *et al.*, 1993). By contrast, two smaller studies by Caparros-Lefebvre *et al.* (1993, 1999) showed some improvement in LID with chronic VIM stimulation. In both reports, however, the authors attributed this unexpected finding to spread of electrical stimulation outside of the VIM proper, caused by the orientation or trajectory of the DBS electrode, and involving either the ventral oralis or centromedian-parafascicular nuclear groups.

In summary, surgical manipulations of the VIM thalamus appear to have little direct effect on LID. This follows from the role of the VIM as the cerebellar-receiving component of the ventrolateral thalamus, which places it outside of the classically described pallidothalamic pathway implicated in dyskinetic activity. Conversely, the VOA/VOP complex is the thalamic representative within this pathway, and lesioning or stimulation of the VOA/VOP seems to alleviate LID, although this observation is not supported by a large amount of clinical data.

## B. Globus Pallidus Interna

### 1. *Pallidotomy*

Leksell's posteroventral pallidotomy was revived by Laitinen *et al.* (1992) and has been shown to cause an immediate and permanent reduction on LID contralateral to the side of the lesion, as well as a significant positive impact on off period motor performance (Guridi *et al.*, 2008). In our experience, all three forms of LID (i.e., peak dose, biphasic, and off-period dystonia) are ameliorated by pallidotomy (Alkhani and Lozano, 2001; Lang *et al.*, 1997). In 2001, we published a systematic review of the literature on pallidotomy from 1992 onward, and found across 85 articles reporting on nearly 2000 patients that, on average, contralateral dyskinesias were reduced by 86.4% at 1 year, and further that the maximum anti-dyskinetic benefit was sustained for roughly 4 years (Alkhani and Lozano, 2001). Longer-term follow-up by ourselves (Kleiner-Fisman *et al.*, 2010) and others (Fine *et al.*, 2000; Ondo *et al.*, 2006) has shown that the anti-dyskinetic effect may remain for at least 10 and in some cases up to 13.5 years (Hariz and Bergenheim, 2001). Efficacy against LID following pallidotomy does not require a reduction in L-dopa dose (Guridi *et al.*, 2008).

The precise location of the pallidotomy lesion within the GPi and its impact on efficacy in LID has been a matter of some debate. In general, lesions of the anterodorsal GPi can mimic the effects of L-dopa, producing improvements in rigidity and akinesia, but potentially inducing or worsening dyskinesias (Follett, 2004). Posteroventral lesions are better at relieving LID, but may worsen akinesia. We reported that even within the posteroventral GPi, there is a variable response to lesioning along its anteromedial to posterolateral axis: lesions situated more anteriorly and medially are better at reducing on period dyskinesias and off period rigidity, centrally placed lesions improve akinesia, and posterior lesions produce greater tremor reduction (Gross *et al.*, 1999). These findings may argue for the importance of MER prior to lesioning in order to precisely define the pallidotomy target. By and large, bilateral pallidotomy is perfomed infrequently because of an increased risk of complications, specifically dysarthria and cognitive deterioration (Merello *et al.*, 2001).

### 2. *Pallidal DBS*

There is now class I evidence supporting high frequency DBS of the GPi as superior to best medical management in improving off period motor symptoms, LID, and overall quality of life in patients suffering from moderate to severe PD (Bronstein *et al.*, 2011; Moro *et al.*, 2010; Weaver *et al.*, 2009). This evidence represents the confirmation of several previous non-randomized studies that reported a significant and stable anti-dyskinetic effect with both unilateral (Visser-Vandewalle *et al.*, 2003) and bilateral GPi DBS (Ghika *et al.*, 1998; Loher *et al.*, 2002; Obeso *et al.*, 2001; Rodriguez-Oroz *et al.*, 2005; Volkmann *et al.*, 2004). A review of these published studies reveals an average reduction in LID of 64–78% in the short term, with a more variable reduction of between 28–64% at long-term follow-up.

Similar to the pallidotomy experience, some studies examining stimulation in the acute setting confirm the existence of distinct sites in the GPi at which DBS may produce opposing effects, with ventral pallidal stimulation tending to antagonize L-dopa effects and dorsal stimulation mimicking L-dopa (Bejjani *et al.*, 1997; Krack *et al.*, 1998b). This has led some authors to advocate stimulation of the middle portion of the GPi between these two extremes, which may produce a suitable compromise between improvements in the cardinal symptoms of PD and amelioration of LID (Follett, 2004).

### 3. *Mechanisms of Action*

The proven efficacy of pallidotomy and pallidal DBS has lent some support to the classic concept that an intact pallidothalamic tract is a prerequisite for the development of LID, and that dyskinetic activity can be abolished by disrupting

this tract. As we have seen, however, a reduction in the firing rate of the GPi, whether due to a lesion or electrical stimulation, sets up a paradox because such a decrease in firing rate—at least according to the rate model of basal ganglia function—should release the thalamus from inhibition with the resultant production of unwanted or excessive movements.

In response to this paradox, a more complex explanation has evolved. Some authors now believe that chronic L-dopa use leads to the creation of an abnormal pattern of activity that originates in the basal ganglia and is eventually transmitted to the cortex (Guridi *et al.*, 2008). The net result of this altered activity is a release of dyskinetic movements. These movements are indeed correlated with reduced firing rates in the GPi but also more subtle neurophysiological changes including alterations in firing synchrony between neuronal subpopulations, interspike interval, and bursting activity (Guridi *et al.*, 2008; Lozano *et al.*, 2000; Obeso *et al.*, 2000). The anti-dyskinetic activity of pallidotomy may be due to its ability to correct these abnormal patterns by obliterating those GPi neurons that express or help sustain them. Similarly, pallidal DBS likely affects the same sub-populations of GPi neurons, although the mechanisms of action underlying DBS altogether are still incompletely understood. The simplistic notion that DBS produces a depolarizing blockade within target neurons is being replaced by experimental evidence showing that the mode of action of DBS varies according to target (Benabid *et al.*, 1998; Benazzouz and Hallett, 2000). In the GPi, DBS may in fact result in a complex reshaping of the temporal structure of neuronal activity. Work done in MPTP-treated monkeys undergoing acute GPi stimulation has revealed a stereotyped triphasic response time-locked to each individual train of stimuli: a period of initial excitation is followed by inhibition, and then followed by a second excitation, with a subtle overall decrease seen in firing rate averaged over the entire nucleus (Bar-Gad *et al.*, 2004).

## C. Subthalamic Nucleus

### *1. Subthalamotomy*

In experimental parkinsonian animals, lesioning of the STN has been shown to improve motor function (Bergman *et al.*, 1990; Blandini *et al.*, 1997; Guridi *et al.*, 1996). In humans, subthalamotomy has been used only sparingly in PD patients, owing to the risk of inducing contralateral hemiballism or hemichorea (Barlas *et al.*, 2001; Guridi *et al.*, 2008). A handful of studies have reported on this limited experience (Alvarez *et al.*, 2001, 2005, 2009; Barlas *et al.*, 2001; Patel *et al.*, 2003; Su *et al.*, 2002; Vilela Filho and Da Silva, 2002). Indeed, there is a low but real incidence of hemiballism/chorea, on the order of 15%, which tends to be self-limited but which may be permanent and require further treatment in up to

50% of these patients (Alvarez *et al.*, 2009). Nevertheless, there is also a definite improvement in motor function. With the exception of Alvarez *et al.* (2001), LID is consistently and significantly reduced at medium- or long-term follow-up in the range of 50–74.2%. Interestingly, the initial paper by Alvarez *et al.* (2001) was the only series in which the dose of L-dopa was not reduced, compared to a mean dose reduction ranging from 34–47% in the other series, including two more recent studies by the same group (Alvarez *et al.*, 2005, 2009). Consequently, the existence of a direct anti-dyskinetic effect of STN lesioning—or whether the effect is secondary to a post-operative reduction in L-dopa intake—is still a matter of debate. Furthermore, we still lack a satisfactory explanation as to why a lesion within the STN may on the one hand reduce LID while on the other produce an occasional paradoxical increase in hyperkinetic movements such as hemiballism/chorea.

### 2. *STN DBS*

DBS of the STN is the most commonly chosen procedure used to treat PD. There is now evidence from randomized, controlled trials for the efficacy of STN DBS against the cardinal symptoms of PD as well as overall quality of life (Bronstein *et al.*, 2011; Follett *et al.*, 2010; Weaver *et al.*, 2009). These positive effects are sustained up to at least 5 years (Bronstein *et al.*, 2011; Gervais-Bernard *et al.*, 2009; Krack *et al.*, 2003; Moro *et al.*, 2010; Rodriguez-Oroz *et al.*, 2005; Schupbach *et al.*, 2005). The motor improvements seen with STN DBS are essentially equivalent to those brought on by DBS in the GPi (Anderson *et al.*, 2005; Burchiel *et al.*, 1999; Follett *et al.*, 2010). However, stimulating the STN may be associated with an increased risk of new or worsened psychiatric or cognitive side effects (Bronstein *et al.*, 2011; Follett *et al.*, 2010). Conversely, STN DBS consistently allows for a post-operative reduction in total L-dopa dose (Follett *et al.*, 2010).

STN DBS produces a significant and sustained reduction in LID, essentially equivalent to that achieved by GPi stimulation, but largely proportional to the reduction in L-dopa dose (Guridi *et al.*, 2008; Herzog *et al.*, 2003). Guridi *et al.* (2008) reviewed the largest case series of STN DBS published until 2008 and found a range in mean dyskinesia reduction of 46.4–85%, accompanied by a mean daily L-dopa reduction of 22–70%. As observed with GPi DBS, Krack *et al.* (1999) found that STN DBS improves the entire spectrum of LID including peak dose, biphasic, and off period dyskinesias. Interestingly, and in keeping with the finding of hemiballism/chorea seen with subthalamotomy, many patients who undergo STN DBS develop choreiform movements in the immediate post-operative period which are similarly transient and may even predict a favorable surgical outcome (Houeto *et al.*, 2003).

### 3. *Mechanisms of Action*

The improvement in LID in PD patients undergoing subthalamotomy is likely multi-factorial. First and most importantly, subthalamotomy significantly reduces daily L-dopa requirements. According to Guridi *et al.* (2008), this probably results in a shift in the dose-response curve away from the induction of LID; LID may still be induced but only by administering a higher dose of L-dopa or exposing patients to a period of L-dopa resensitization. This indirect mechanism may be complemented by a more direct effect of STN lesioning, helping to explain why some authors have noted an improvement in LID that precedes the actual reduction in L-dopa (Su *et al.*, 2002). A reasonable hypothesis is that a distinct subsection of the STN may contribute to the generation of an abnormal dyskinetic pattern of activity (Guridi *et al.*, 2008). Accordingly, experimental evidence in the MPTP monkey shows an association between LID and increased metabolism in the ventromedial (i.e., limbic) portion of the STN (Mitchell *et al.*, 1985). In humans, the 4–10 Hz oscillations associated with LID also predominate in the ventral STN (Alonso-Frech *et al.*, 2006). Conceivably, a lesion in the ventral STN could disrupt a necessary component of the circuit subserving LID, resulting in a clinical anti-dyskinetic effect.

Another possible explanation relates to a relative stabilizing effect of STN lesioning on fluctuations in the basal ganglia. Recall that subthalamotomy may initially induce abnormal hemiballism/chorea, which is frequently self-limited. Following this initial hyperkinetic period, there is good data to suggest that the basal ganglia transitions to a state of "functional normalization" (Guridi *et al.*, 2008). In both parkinsonian rats treated with 6-hydroxydopamine (6-OHDA) or MPTP-treated monkeys, metabolic markers of neuronal activation, as well as markers of GABAergic inhibition such as mRNA expression of glutamic acid decarboxylase, reach a steady state following subthalamotomy (Blandini *et al.*, 1997; Guridi *et al.*, 1996). The ultimate manifestation of this new equilibrium is to minimize the impact of L-dopa in the parkinsonian state, shortening its effects via the so-called "wearing off" model and resulting in a clinical reduction in motor fluctuations (Marin *et al.*, 2004). In essence, the basal ganglia become buffered against the prodyskinetic effects of L-dopa.

Any of the putative mechanisms that apply to subthalamotomy are also suitable explanations for the efficacy of STN DBS against LID. Indeed, a reduction in L-dopa medication following DBS is unequivocally linked to a proportional decrease in dyskinesias. In addition, stimulating the STN may dampen pathological fluctuations in the basal ganglia, which some authors have likened to the effect of a continuous administration of L-dopa (Guridi *et al.*, 2008; Obeso *et al.*, 2000), although it has been well established that DBS does not actually alter dopamine release *per se* (Abosch *et al.*, 2003). More recently, there has been some evidence for a direct anti-dyskinetic effect of STN related to stimulation of structures in the

dorsal STN region as opposed to within the STN proper. Alterman *et al.* (2004) published a case where the dorsal relocation of a correctly placed STN electrode produced immediate and sustained relief of contralateral LID. Moreover, some now argue that the dorsal subthalamic area, which includes the zona incerta and the lenticulus fasciculari (a component of the pallidothalamic pathway), may in fact be the real target of STN DBS (Plaha *et al.*, 2006; Saint-Cyr *et al.*, 2002). If this is the case, then DBS of this region may contribute to improvements in LID through a disruption of the classic pallidothalamic connection.

Another possibility is that DBS in the STN may actually drive, as opposed to inhibit or stabilize, neuronal or axonal outflow activity within the basal ganglia, and in so doing, circumvent the abnormal synchronization seen in PD (Guridi *et al.*, 2008). Garcia *et al.* (2003) have called this the "dual effect" of high frequency stimulation in the STN: there is first a suppression of spontaneous activity and next the induction of a new therapeutic pattern of activity at a characteristically higher frequency. Part of this effect is a reduction in the overall presence of $\beta$-band activity (Brown, 2003).

## IV. Surgical Approach to the Patient With LID

### A. Selection of the Patient and Target

Surgery is the only current treatment that can effectively and simultaneously treat both the motor symptoms of PD as well as LID (Guridi *et al.*, 2008; Toda *et al.*, 2004). Consequently, the selection of patients for surgical intervention to treat LID is invariably coupled to a consideration of the efficacy of surgery against the cardinal features of the parkinsonian state, with rare exceptions. Furthermore, the safety, efficacy, and reversibility of DBS make it the contemporary method of choice; the role for lesioning procedures is becoming increasingly limited (Bronstein *et al.*, 2011; Esselink *et al.*, 2006; Gross, 2008; Starr *et al.*, 1998). In particular, the paucity of evidence supporting the efficacy of thalamotomy against LID and the risk of hemiballism/chorea associated with subthalamotomy have all but eliminated these two procedures from the movement disorder surgery armamentarium. Pallidotomy is still a reasonable option to control contralateral LID in the PD patient with predominantly unilateral dyskinesias who is either a prohibitive medical risk for DBS, wants to avoid implanted hardware, is at elevated risk of infection, has a history of recurrent DBS infection with a previously implanted system, or is unwilling to commit to long-term programming (Bronstein *et al.*, 2011). These indications must be weighed against the small but real risk of a permanent neurological deficit resulting from a

mistargeted lesion, the occasional need for multiple procedures to improve efficacy, and the increased risk of side effects if bilateral pallidotomy is attempted (Bronstein *et al.*, 2011; Gross, 2008).

The ideal candidate for DBS to treat LID has functionally disabling dyskinesias in the setting of moderate to severe PD, accompanied by severe motor fluctuations, and has exhausted all appropriate medical options. Ideally, LID should be a dose-limiting side-effect of L-dopa therapy (Follett, 2004), but patients should still derive benefit from L-dopa, demonstrated by at least a 30% improvement in motor scores on part III of the Unified Parkinson Disease Rating Scale (UPDRS) in response to a standard L-dopa challenge (Charles *et al.*, 2002; Kleiner-Fisman *et al.*, 2006) The only firm exclusionary criterion for DBS is dementia, although active psychiatric conditions are a relative contraindication and should aim to be brought under control before surgery (Bronstein *et al.*, 2011).

To date, head-to-head studies have not shown a significant difference in efficacy against either LID or cardinal motor symptoms between pallidal and STN DBS (Burchiel *et al.*, 1999; Bronstein *et al.*, 2011; Follett *et al.*, 2010). As a result, target selection must be individualized to the patient. Several factors may play a role in choosing the right target for the right patient. Each patient's unique risk profile for side effects, personal preferences regarding the frequency and dose of medication, as well as surgeon experience and comfort must all be considered. If a withdrawal or reduction of medication is the desired goal of surgery, then STN DBS is the clear choice. STN DBS is also the best option in patients previously treated with a pallidotomy (Ondo *et al.*, 2006). Conversely, there is evidence that STN DBS may be complicated by adverse alterations in mood or impulsivity, leading in extreme cases to increased suicide risk (Voon *et al.*, 2008). In addition, cognitive and executive frontal lobe function may be impaired by targeting the STN (Saint-Cyr *et al.*, 2000). The GPi therefore becomes the preferred target in patients with pre-existing psychiatric or cognitive conditions.

### B. Technical Considerations

The stereotactic surgical techniques used to perform lesioning procedures or to implant DBS systems are variable from center to center, in constant evolution, and the subject of an extensive body of literature. Consequently, we will not exhaustively describe these techniques further in this review. Nevertheless, there are some technical considerations unique to performing DBS for LID, which are worth mentioning.

Typically, implantation of DBS electrodes is done after patients have been in the off-medication state for several hours, usually following an overnight period free of L-dopa consumption. This gives the best opportunity to avoid motion artifact caused by dyskinesias which may corrupt pre-operative imaging, alter

intra-operative MERs, or cause a patient to slip out of pin fixation in the stereotactic frame (Follett, 2004). These advantages are offset, of course, by the inability to document a reduction in LID using intra-operative stimulation, although macroelectrode stimulation should still be used in the standard fashion to confirm proper electrode position by the absence of stimulation side effects and, frequently, by stimulation-dependent improvement in parkinsonian symptoms.

To date there is no definitive evidence that refining the target with MER improves outcome or, conversely, sacrifices safety in either lesioning or DBS procedures of the GPi and STN (Sierens *et al.*, 2009). However, with the notable exception of some centers (Hariz, 1999; Hariz *et al.*, 2004), MER is a routine and nearly universally applied technique in functional movement disorder surgery (Ondo and Bronte-Stewart, 2005). Moreover, in the setting of LID treatment there may be an added incremental benefit to using MER in targeting the STN because recordings can help identify the dorsal (i.e., theoretically anti-dyskinetic) border of the nucleus (Alterman *et al.*, 2004).

### C. DBS Programming for LID

In a review of North American practices, initial programming of internalized DBS systems was performed on average 18 days after electrode placement but ranged from 1–90 days (Ondo and Bronte-Stewart, 2005). A detailed analysis of the various stimulation parameters and adjustment options is outside the scope of this review; for an overview of basic DBS programming algorithms, the reader is referred to Volkmann *et al.* (2006). In brief, there are two general approaches to DBS programming in the setting of LID (Follett, 2004; Kumar, 2002). The first and more common approach takes into account that the primary aim of most DBS procedures in PD is to improve cardinal off-period motor symptoms. This approach argues that programming should be done with the patient in the off-medication state following an overnight washout (Volkmann *et al.*, 2006). Once the optimal relief of these symptoms is achieved, the patient can then begin taking medication and be monitored for dyskinesia. Dyskinesias will usually manifest or be worse in the afternoon, after the patient has taken several doses of L-dopa. If no dyskinesias are observed in the on-state, then the stimulation parameters require no further adjustment. If dyskinesias are present, then programming modifications should be undertaken, but again only during the off period, to ensure that off-medication motor benefit is preserved (Kumar, 2002). The second approach argues that performing all programming in the on-medication state provides immediate feedback on the anti-dyskinetic effect of DBS, in turn permitting immediate reprogramming and the greatest opportunity for maximally reducing LID (Follett, 2004). This approach risks exacerbating off-medication motor symptoms as well as off-period dyskinetic activity.

In theory, the selection of active DBS contacts ought to take into account the most anti-dyskinetic portion of the target area. As we have seen, for pallidal DBS this is the posteroventral GPi usually corresponding to the deepest contacts; in the STN it may be the dorsal region corresponding to the most superficial contacts. In practice, considerable trial and error is needed to determine the optimal electrode configuration in each patient (Kumar, 2002; Volkmann *et al.*, 2006). Striking a balance between achieving anti-dyskinetic effects and avoiding antagonism of beneficial motor effects is critical. In the GPi, where DBS has a proven direct anti-dyskinetic effect, some authors believe that this argues in favor of using contacts situated in the central portion of the nucleus (Bejjani *et al.*, 1997; Krack *et al.*, 1998b; Kumar, 2002). In the STN, by contrast, where the direct anti-dyskinetic effect is of uncertain importance, the optimal electrode configuration is one which permits the maximum reduction in L-dopa dose without loss of motor function (Krack *et al.*, 2002).

## V. Conclusion

Treatment with L-dopa produces an unquestioned improvement in the motor function and quality of life of patients with PD. Unfortunately, a substantial number of these patients are functionally disabled by LID with the subsequent need for modifications in medical therapy. Modern day stereotactic surgical lesioning procedures and DBS are effective treatments against LID and are simultaneously and predictably effective against the cardinal features of PD as well. As the surgical treatment of LID has undergone an evolution from early empirical procedures, so too has our understanding of basal ganglia physiology. At the same time, some of the paradoxical effects of surgery for LID have confounded attempts to model the basal ganglia, ushering in new ideas about its oscillatory state in PD and in response to chronic L-dopa therapy. Going forward, further basic and clinical research is going to be necessary to refine the surgical treatment of LID, whether through the improvement of existing techniques, or by identifying new targets for intervention.

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# CLINICAL AND EXPERIMENTAL EXPERIENCES OF GRAFT-INDUCED DYSKINESIA

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Clinical trials evaluating transplantation of fetal tissue for the treatment of Parkinson's disease identified the unexpected side effect of abnormal movements in the 'off' L-DOPA state. Termed graft-induced dyskinesia (GID), various hypotheses have been put forward as to their cause but unfortunately the significant differences in clinical trial protocols and lack of a truly representative animal model has hindered the search for a conclusive basis for their appearance. Likely causative factors have been identified through careful examination of patient data and the use of amphetamine-induced dyskinesia in a rodent model of Parkinson's disease. New trials being planned in Europe hope to avoid GID, whilst maximizing on the functional benefit that can be afforded by this treatment approach but questions still remain as to the underlying mechanism.

## I. Introduction

For most patients, L-DOPA or dopamine agonist pharmacotherapy produces very effective relief of some motor symptoms in the early stages of Parkinson's disease. As the disease progresses, dopamine agonists and other adjunctive agents become increasingly less able to counteract the symptoms and there is a greater need for L-DOPA to form a major part of their pharmacotherapy. Both disease progression associated with ongoing degeneration of the nigrostriatal dopaminergic pathway and the increasing doses of L-DOPA required to control the symptoms

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00007-9




0074-7742/10 $35.00

contribute to the risk of developing abnormal involuntary movements known as L-DOPA-induced dyskinesia (LID) (Grandas *et al.*, 1999). The significant impact of LID on quality of life, and the fact that these abnormal movements limit the quantity of L-dopa that can be administered, has driven the need to understand the mechanisms underlying LID and the search for anti-dyskinetic agents. Fraught with difficulties, putative agents have demonstrated clear potential in pre-clinical models but then failed to translate into successful clinical trials, either being ineffective in the treatment of the LID in patients, or simultaneously worsening the parkinsonism (reviewed in Fox *et al.*, 2006; Lane and Dunnett, 2008). L-DOPA remains the most effective treatment especially in the latter stages of the disorder but given these problems, alternative treatment strategies have also been considered. One strategy is the transplantation of dopamine producing cells into the putamen which has successfully ameliorated motor symptoms for some patients with idiopathic PD and also patients with PD resulting from the accidental self-administration of MPTP (Madrazo *et al.*, 1991; Freed *et al.*, 1992; Lindvall *et al.*, 1994; Peschanski *et al.*, 1994; Kordower *et al.*, 1998). However, the implementation of this technique in two double-blind clinical trials revealed a new form of intractable dyskinesia caused by the graft in the absence of L-DOPA. This chapter will detail what is known to date of these "graft-induced dyskinesia" (GID) in terms of their clinical presentation, hypotheses of their cause, the development of animal models and how new clinical trials are being designed with a view to avoiding them.

## II. Transplantation for Parkinson's Disease

More than three decades ago, research groups in Europe and the United States began to work on the concept that the ectopic implantation of catecholaminergic neurons into the putamen would replace the dopamine lost through the disease process and may alleviate the motor symptoms of Parkinson's disease. In support of this, early experiments in the 6-OHDA lesioned rat demonstrated that the transplantation of adrenergic or developing fetal dopaminergic tissue into the striatum of hemiparkinsonian rodents would ameliorate motor deficits (Backlund *et al.*, 1985; Bjorklund *et al.*, 1980; Dunnett *et al.*, 1981; Freed, 1983; Nadaud *et al.*, 1984). Clinical trials followed and whilst there were significant benefits to the autologous transplantation of the patients own adrenal gland cells, avoiding the need for immunosuppression and therefore related complications, this approach only produced mild clinical benefit (Backlund *et al.*, 1985; Lindvall *et al.*, 1987). The only study reporting on the clinical neuropathology of an adrenal transplant recipient failed to find survival of chromaffin cells 16 years post-transplantation (Kompoliti *et al.*, 2007).

The earliest open label trials carried out with the allogeneic transplantation of fetal cells demonstrated that the strategy of obtaining dopaminergic neurons from 6 to 9 weeks embryos following elective surgical terminations of pregnancy did have the potential to provide significant clinical benefit (Freed *et al.*, 1992; Lindvall *et al.*, 1990; Madrazo *et al.*, 1991). Functional recovery could be achieved along with the reduction, and in some cases complete cessation of anti-Parkinson's medication, in itself alleviating the dyskinesia associated with L-DOPA administration (Hagell *et al.*, 1999). In contrast to the autologous adrenal cell transplants, fetal cell transplant pathology of a similar survival time shows the clear maintenance of the graft and high expression of tyrosine hydroxylase indicative of sustained dopamine production (Kordower *et al.*, 2008; Li *et al.*, 2008). This is consistent with maintained functional improvement over several years (Politis *et al.*, 2010).

On the tails of the success of the open label trials, there was a drive to follow the pharmaceutical rationale of double-blind, placebo-controlled trials. While the appropriateness of such a trial in long-term surgical therapies such as transplantation may be (and indeed has been) debated (R. Barker, personal communication), the outcome of two NIH sponsored trials (Denver-Colombia and Tampa-Mt Sinai trials) to prove the efficacy of transplantation threw the field into disarray. Not only did they both report an efficacy far below that which was predicted by the open label trials, there was also the development of a novel form of dyskinesia, abnormal involuntary movements present in clinically defined 'off' (Freed *et al.*, 2001; Olanow *et al.*, 2003). These movements, now termed 'graft-induce dyskinesia' (GID), were then also identified in a retrospective analysis of patients transplanted in a successful open-label trial (Lund-London trial) (Hagell *et al.*, 2002). There was a large amount of negative publicity generated, and the result was a mutual consensus to cease transplanting patients until these issues of efficacy and GID could be resolved.

## III. The Clinical Phenomena of GID

In 1999, Greene and coworkers published the first abstract reporting on GID in the patients transplanted in the Denver-Colombia study (Greene *et al.*, 1999). Fuller details were reported in the subsequent article in the New England Journal of Medicine, reporting that "dystonia and dyskinesia developed in five patients (15%) and persisted after a substantial reduction in, or elimination of therapy with dopamine-agonist drugs" (Freed *et al.*, 2001; Greene *et al.*, 1999). The movements were described variously as being either severe cranial dystonia, persistent dyskinesia in the arm or generalized dyskinesia (Freed *et al.*, 2001). Two years later the Tampa-Mt Sinai double-blind NIH-funded trial reported that 57%, 13 out of

23 patients, developed abnormal movements in clinically defined 'off' in which the lower extremities, namely leg and hip, were the most affected with "repetitive stereotypic alternating contractions of flexor and extensor muscle groups at a relatively low frequency" (Olanow *et al.*, 2003). The Lund-London patients (6 out of 14, 43%) reviewed by video, presented with concurrent hyperkinesia and dystonia, also predominantly in the lower limbs with "ballistic repetitive stereotypic movements" (Hagell *et al.*, 2002). At this point it is also worthy of mention that the development of GID in other open label trials was not explicitly looked for or clearly evaluated; however, there were mentions of delayed onset changes in dyskinesia in one Swiss open-label trial report and an earlier open-label trial by the Tampa-Mt Sinai collaboration (Graff-Radford *et al.*, 2006; Jacques *et al.*, 1999). Nevertheless, the above descriptions highlight differences in the expression of GID elicited in each trial. In the Tampa-Mt Sinai and Lund-London groups the behaviors were typically in the lower extremities and stereotypic in nature, conversely in the Denver-Colombia study, more axial and facial and upper body dystonia were reported. This could simply be a facet of the location of the graft in the somatotopically organized putamen or a directly comparable difference in symptomatology. Despite these phenotypic differences, one important consistency between all groups was that patients suffering from GID also benefited from the graft with a degree of improvement in motor function, albeit slight in the double blind trials (Hagell *et al.*, 2002; Olanow *et al.*, 2009). Whilst some patients improved significantly without the development of GID, these data support the assumption that the behaviors are a direct consequence of the transplantation procedure and only develop when there is a surviving graft-producing dopamine.

Aside from the obvious difference in double-blind versus open-label trials, the transplantation protocols varied in many parameters including the selection of patient cohorts, immunosuppression regimes, and measures used in the assessment of functional outcome. This is excellently reviewed by Winkler *et al.* (2005), but in summary, the features potentially critical to GID were firstly in the areas of tissue preparation. The Denver-Colombia trial implanted "noodles" of cells cultured for 4 weeks prior to transplantation in a frontal approach. The Tampa-Mt Sinai trial transplanted solid pieces of tissue either freshly obtained or stored for 2 days (Tampa), whilst Lund-London grafted cell suspension either fresh or occasionally up to a week when necessary to garner enough tissue. The number of ventral mesencephalon used per transplant varied from 1 to 7 across the trials and immunosuppression regimes ranged from none administered in the Denver-Colombia trial, with up to 4 years of continuous cyclosporine and steroid use in the Lund/London trial. Other variations that could also be significant were in the mean age of patients, disease duration, their UPDRS motor score in 'off' and medication in L-DOPA equivalents. Drawing any firm conclusions for the basis of GID from this varied profile has been impossible.

## IV. Animal Models of GID

A major difficulty in studying GID is that they are purely clinical phenomena, and only a few patients globally are known to have developed them. Given that this was not an anticipated consequence of transplantation, one possibility was that the behaviors had simply been overlooked, especially as clinical reports were diverse and generally described mild dyskinesia. Prior to the clinical trial reports, a plethora of animal studies had been performed in parkinsonian rodents and primates none of which reported any incident of spontaneous abnormal movements. Following the publication of the clinical findings and recognition of the potential impact of GID on the field, transplantation studies were carried out in the 6-OHDA lesioned rat and MPTP-treated primate specifically to look for spontaneous GID. Two rodent studies in Lund, Sweden, closely examined 6-OHDA lesioned rats' behavior post-transplantation (Carlsson *et al.*, 2006; Lane *et al.*, 2006). The concept was to replicate the experience of the patient as closely as possible; patients were in the later stages of the disease treated with L-DOPA and typically had developed LID. Therefore, in the animal model 6-OHDA lesions were used, a severe unilateral lesion producing 95–99% depletion of nigrostriatal dopamine, and rats were pre-treated with L-DOPA to generate LID. Following transplantation with ventral mesencephalon from rat embryos at embryonic day 14, an approximate correlate of the 6–9 weeks human embryos used in patient trials, no spontaneous abnormal movements were observed. Mild stressors were applied to the rats, tail pinch for example, in an attempt to trigger spontaneous dyskinesia but to no avail (Carlsson *et al.*, 2006; Lane *et al.*, 2006). Mild, sporadic dyskinesia in the absence of L-DOPA was observed in one of these, and one other reported study but these were too occasional, inconsistent, and easily interpretable as normal behaviors to use as a robust model (Lane *et al.*, 2006; Vinuela *et al.*, 2008). A large-scale primate study was also conducted but spontaneous dyskinesia was similarly elusive (Redmond *et al.*, 2008). An important and unexpected outcome was in the rodent studies. Amphetamine is commonly used to crudely assess the extent of the 6-OHDA lesioned (triggering an ipsilateral rotational response) and to test the success of transplantation (contralateral rotations in a distinctive biphasic pattern (Bjorklund *et al.*, 1980; Dunnett *et al.*, 1983; Herman *et al.*, 1993; Torres and Dunnett, 2007)). In testing the transplanted rats with amphetamine, a proportion of the rats began to display abnormal movements similar in nature to the L-DOPA-induced AIMs (described in Johnston and Lane, this volume). Whilst not being spontaneous, these amphetamine-induced AIMs have parallels with GID that support its use a model for the movement disorder. They are only present following transplantation; if the graft is rejected, the response to amphetamine reverts back to a pre-transplantation state with no dyskinesia (Lane *et al.*, 2008). Moreover they are present in the *absence* of L-dopa approximating the clinically defined 'off' and

prolonged withdrawal of L-DOPA in patients. However, it is noteworthy that some transplanted patients were given amphetamine as part of an imaging study and it did not appear to evoke or exacerbate GID (P. Piccini, personal communication). These rodent abnormal movements in response to amphetamine develop over 12–16 weeks post-transplantation despite the almost immediate reversal of the rotational response (Lane *et al.*, 2006). This suggests that the mechanisms are not simply as a result of dopamine release, but may relate to the way the graft develops. In general, and in agreement with clinical studies, rats with GID have improved motor function and reduced LID when L-DOPA is administered suggesting that they only occur with a functionally effective transplant. A couple of years prior to these studies, a US group had identified behavioral changes in transplanted animals in response to L-DOPA. They first reported increased forelimb hyperkinesia and in a later study describe the development of L-DOPA-induced "forelimb-facial stereotypy" and "forepaw tapping" that only develops post-transplantation (Maries *et al.*, 2006; Soderstrom *et al.*, 2008; Steece-Collier *et al.*, 2003). These behaviors, induced by L-DOPA, are even further removed methodologically from clinical GID than amphetamine-induced behaviors but provide strong indications that even distributions of graft tissue and immune reactions to the graft can influence motor function (Maries *et al.*, 2006; Soderstrom *et al.*, 2008).

## V. Understanding the Cause of GID

Each clinical trial reporting GID has identified potential influential factors in their analyses. The first hypothesis, put forward by the Denver-Colombia study, was that "continued fiber outgrowth from the transplant has led to a relative excess of dopamine"[sic] (Freed *et al.*, 2001). [$^{18}$F]DOPA PET scans quickly negated this hypothesis demonstrating that dopamine levels were below that of a healthy caudate putamen. However, these scans did reveal that some grafts were not homogenous in distribution and that "hotspots" of dopamine within the transplanted putamen were only present in patients with GID (Ma *et al.*, 2002). This sounded like a plausible explanation, uneven distribution of the cells causing intense areas of dopamine release, which could trigger excessive, uncontrolled motor responses. Unfortunately similar scans from patients in other trials did not validate this hypothesis, failing to find any evidence of "hotspots" in other transplanted patients (Hagell *et al.*, 2002). Moreover, reports from the Tampa-Mt Sinai cohort of GID patients, with a more stereotypic phenotype, likened the movements to the end-of-dose dyskinesia observed with L-DOPA administration, as opposed to the more classic peak-dose LID. They suggest, in contrast to the Denver-Colombia hypothesis, that low levels of dopamine are responsible for GID implicating suboptimal

grafts that produce insufficient dopamine to normalize striatal function and instead intermittently activate the sensitized dopamine receptors (Olanow *et al.*, 2009). Preclinical studies in the 6-OHDA lesioned rats evaluated the consequence of graft size but failed to find any association with amphetamine-induced dyskinesia (Lane *et al.*, 2006). Interestingly, rat studies suggest that it may be more to do with the location of striatal reinnervation and that more caudal and lateral areas of the caudate putamen may be associated with the expression of amphetamine-induced dyskinesia (Carlsson *et al.*, 2006; Lane *et al.*, 2010b). Maries and coworkers tried to reproduce hotspots in the graft by creating concentrated dopamine cell deposits in the rat striatum (Maries *et al.*, 2006) but this study focused on the consequences of the transplant on LID rather the GID. Nevertheless, they did find that while focal grafts reduced LID they did so less consistently than widespread grafts and that there was an increase in mild stereotypies of the forelimb and orolingual area. These studies contribute to the clinical findings that best functional recovery will be obtained with even, widely innervating grafts.

One of the more recent hypotheses has postulated a role for the neurotransmitter serotonin in the development of GID, partly in response to findings of its significant role in LID (Carta *et al.*, 2008). As dopaminergic terminals degenerate, there are insufficient numbers to readily convert the exogenously administered L-DOPA into dopamine. Serotonergic terminals in the striatum possess the necessary cellular machinery to carry out this process and appear to take up L-DOPA, synthesize, store, and release dopamine (Maeda *et al.*, 2005; Tanaka *et al.*, 1999). Given that the appropriate feedback systems can no longer function as they would in a dopaminergic terminal, dysregulated dopamine release may contribute significantly to the dyskinesia in advanced stages of the disease (Carta *et al.*, 2010). The significance of the issue in GID is that the developing dorsal raphé is located immediately caudal to the ventral mesecephalon. Depending on the dissection parameters, some serotonin neurons may be included in the VM tissue dissected and transplanted. There is a limited knowledge of how much serotonin may have been transplanted into patients in previous clinical trials as serotoninergic neurons have proved difficult to identify conclusively in the post-mortem cases that have been analyzed. However, this analysis has been successfully performed in five patients from one transplantation study in Nova Scotia, Canada identifying a high proportion of serotonin neurons in the grafts of five patients (Mendez *et al.*, 2008). Furthermore, [$^{11}$C]DASB PET scans of serotonin transporter levels in the caudate putamen of two patients transplanted in Lund identified levels well above those of control subjects and parkinsonian controls (Politis *et al.*, 2010). Critically, the Canadian patients were reported as having no indication of GID whilst the two Lund patients did (Mendez *et al.*, 2008; Politis *et al.*, 2010). Given that patients with successful transplants without GID from the Lund cohort were not scanned, this data is far from conclusive. Rodent studies have found no relationship between GID and serotonergic neuron "contamination" (Lane *et al.*, 2006, 2009) and a

detailed study by Garcia suggests that so long as the number of serotonin neurons does not exceed the number of dopaminergic neurons there is no significant effect (Garcia *et al.*, 2011). This, however, is not the end of the story as both rodent and patient GID studies have suggested that GID are modulated by 5-$HT_{1A}$ agonists but not increased 5-HT levels induced by serotonin selective reuptake inhibition (rodents only) (Lane *et al.*, 2006, 2009; Politis *et al.*, 2010). Care should be taken in the interpretation of the clinical experiment though as buspirone, the 5-$HT_{1A}$ agonist used (a licensed anxiolytic), also functions as a $D_2$-like dopamine receptor antagonist with significant effects on the dopaminergic system (Skolnick *et al.*, 1984). Pre-clinically $D_1$ and $D_2$ receptor antagonists have been found to completely abolish GID; therefore, direct interference through the $D_2$ receptor could also mediate the observed reduction in GID. Nevertheless, these are the only pharmacological studies carried out on GID and open up intriguing questions as to the role of 5-HT. Data from the group of Winkler suggest that functional efficacy may be marginally compromised by the inclusion of 5-HT neurons (Garcia *et al.*, 2011) (although the inverse is found in another study (Carlsson *et al.*, 2007)). This conflicting and potentially coincidental data does not support or condemn the inclusion of 5-HT neurons; therefore, narrow dissections that minimize their incorporation into the graft are justified in future clinical trials, without the need for excessive steps to actively exclude them from the transplant preparation.

Due to the difficulties of procuring 6–9 weeks ventral mesencephalon there was a requirement for some clinical trials to store tissue for short periods of time prior to transplantation (Hauser *et al.*, 1999; Mendez *et al.*, 2000; Olanow *et al.*, 2003). There has also been the addition of factors to improve graft survival, namely lazaroids and GDNF (Brundin *et al.*, 2000; Mendez *et al.*, 2000). While survival factors have not been associated with GID, Hagell *et al.* (2002) reported that the patients who received stored tissue in their trials were among those that developed GID. Storing rodent ventral mesencephalic tissue for 8 days did not increase the likelihood of amphetamine-induced AIMs in the rat model but as shown by others, despite transplanting the same number of cells, dopaminergic neuron survival is significantly reduced (Hebb *et al.*, 2003; Lane *et al.*, 2010b; Nakao *et al.*, 1994; Petersen *et al.*, 2000).

The temporal profile of GID onset varied across the reported studies, a finding that may relate to the variable use of immunosuppression. In the Lund-London trial, there was a significant development or initiation of GID following cessation of immunosuppression, which took place an average 2 years but in some cases up to 4 years post-transplantation (Piccini *et al.*, 2005). This contrasts with the two double-blind NIH studies in which either 6 months or no immunosuppression was given and the abnormal movements were reported as developing and stabilizing in the first 6–12 months following transplantation (Freed *et al.*, 2001; Olanow *et al.*, 2003; Lane *et al.*, 2010a). Multiple donors were used in

all of these studies, an increased pooling might be expected to increase the chances of an inflammatory response to the graft, although there was no difference between the numbers of patients developing GID who received one or four donors (Olanow *et al.*, 2009). It has not been possible to examine the neural tissues of any patients with GID but patients from the same cohorts have shown evidence of inflammation in and around the graft (Kordower *et al.*, 1997). Like the limited clinical insights, pre-clinical studies on inflammation and immunosuppression have been similarly inconclusive but immune responses between the host and grafted tissue may affect synaptic contacts and adversely affect L-DOPA-induced behaviors (Soderstrom *et al.*, 2008).

## VI. Strategies for Dealing with GID

Two or three patients in each experimental cohort of the three trials reporting GID required additional interventions due to the severity of their GID. Amantadine was not successful long-term but the use of DBS had particularly interesting results. Patients in the Denver-Colombia and Lund-London trials reportedly responded well to DBS of the internal globus pallidus (more so than that of the subthalamic nucleus); however, this was only transiently successful in one Tampa-Mt Sinai patient (Cho *et al.*, 2005; Graff-Radford *et al.*, 2006; Herzog *et al.*, 2008; Ma *et al.*, 2002). Conversely, DBS of the subthalamic nucleus did ameliorate movements in all three patients of this trial (Cho *et al.*, 2005). Unfortunately, this disparity again fails to reveal a common mechanism between the three clinical trials. The 5-$HT_{1A}$ agonist buspirone produced a noticeable reduction in GID in the two London patients but is not a possibility as long-term treatment strategy (Politis *et al.*, 2010).

The goal is, and must continue to be, avoiding GID altogether in transplantation alongside producing maximum benefit. However, a consideration when evaluating the potential of transplantation is that despite some patients experiencing relatively prominent GID, their symptoms were better controlled and overall profile of dyskinesia is less severe than had they remained on the best available medication. Their GIDs were significantly less than would be evoked by L-DOPA with a reduction in other motor complications such as "on–off" fluctuations consistent with a reduction in their medication intake. The two patients reported by Politis *et al.* (2010) have disease durations of over 25 years and are still able to walk with ease, read, and communicate.

Controversy surrounds the US led NIH-funded double-blind trials and significant support for transplantation is now more "Europe-centric." Evidence accumulated thus far from examining all the clinical trial data and consideration of the

pre-clinical evidence has been assimilated into the design of an imminent new European clinical trial TransEUro, funded by the EU and led by Dr Roger Barker (Cambridge University). In these multicenter phase II and II/III trials, many of the factors discussed above have been taken into account. Younger, earlier stage Parkinson's patients with no, or low levels of LID will receive grafts of embryonic tissue dissected to minimize the inclusion of 5-HT neurons. They will have long-term immunosuppression and undergo a carefully considered panel of assessments at stages prior to and following transplantation. The results are eagerly awaited and will be major determinant in the future of stem cell therapy for Parkinson's disease.

## VII. Final Considerations

In summary, dyskinesias post-transplantation observed in clinically defined 'off' are described in at least three transplantation clinical trials and can be of a significant magnitude. However, the phenotype varies in each case, the time line of development is different and "curative" sites for DBS are on opposing branches of the basal ganglia circuitry. We may therefore be dealing with an innately variable phenomenon that may in part depend on the location of the dopaminergic deposits in the striatum, serotonin innervation, and duration of immunosuppression. Alternatively are we looking at "GIDisms," similar disorders with different root causes in each trial? We should also bear in mind that GID are considerably milder than LID and are well tolerated by many patients (Hakan Widner, personal communication). Even with GID, patients are significantly better off with a successful transplant than would be predicted if they had continued on their pharmacotherapeutic regime. Whilst the mechanisms are not fully understood, enough information has been ascertained to cautiously move forward with a new clinical trial, the significance of which goes beyond the role of fetal tissue transplants. Success now has repercussions for the use of new sources of cells, whether they are embryonic, adult, or induced pluripotent stem cells, which will supersede the ethically controversial use of fetal tissue.

## Acknowledgments

ELL would like to acknowledge Parkinson's UK, and the European Union (EC contract number 222918 (REPLACES) and EC contract number 242003FP7 (TransEuro) FP7, Thematic priority HEALTH") for their support of studies into GID.

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# TARDIVE DYSKINESIA: CLINICAL PRESENTATION AND TREATMENT

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Tardive dyskinesia (TD) is a common and potentially irreversible side effect of dopamine blocking agents, most often antipsychotics. It is often socially and sometimes also physically disabling. The clinical picture can be divided into orofacial, limb-truncal, and respiratory dyskinesia.

The clinical options to prevent or mitigate TD include psychoeducation, systematic screening, and evaluation of the need for antipsychotics and/or dosages, managementof known risk factors, and switching to an antipsychotic with a lower risk of TD. There is no evidence-based approach for treating existing TD but several clinical interventions can be effective including discontinuing the antipsychotics or reducing the dosage, switching to clozapine, adding an antidyskinetic agent, or applying deep brain stimulation.



DOI: 10.1016/B978-0-12-381328-2.00008-0
0074-7742/10 $35.00

## I. Introduction

Tardive dyskinesia (TD) is a severe neurological side effect of long-term treatment with dopamine blocking agents generally antipsychotics. Antipsychotics are the cornerstone in the treatment of psychotic disorders, particularly schizophrenia, for which long-term antipsychotic treatment is often mandatory. Movement disorders are among the many side effects of antipsychotics. These are divided into acute and tardive movement disorders: the acute variant starts within days or weeks after starting dopamine blocking agents or increasing the dose (or cessation of anticholinergics); tardive movement disorders develop after months or years of treatment with antipsychotics. TD can be potentially irreversible and when it is, the antipsychotic treatment has induced a new syndrome. In mild forms, patients may exhibit psychological problems while severe forms can even cause physical disability.

This chapter presents the clinical picture, pathophysiology, differential diagnosis, prevention, and treatment of TD.

However, two issues need to be discussed first because many subjects in this chapter are based on the assumption that TD is (i) associated with antipsychotics and (ii) is a clinical issue after the introduction of the new (second-generation) antipsychotics.

### A. Is Tardive Dyskinesia Associated With Antipsychotics?

Although most clinicians agree that TD is associated with antipsychotics (it is recognized as a separate category in the DSM-IV), there is still a debate about whether dyskinetic movements are a consequence of antipsychotic use or whether they are closely related to the underlying disease process of schizophrenia itself (Crow *et al.*, 1983; Koning *et al.*, 2010). The hypothesis that the tardive syndromes are related to schizophrenia is based on (i) descriptions of dystonic and dyskinetic features in schizophrenic patients in the pre-neuroleptic era, (ii) substantial prevalence of TD in antipsychotic-naive populations with schizophrenia, and (iii) the absence of a significant association between the duration of antipsychotic use and the presence of TD (Koning *et al.*, 2010). However, several arguments strongly support the relationship between dyskinesia and the use of antipsychotics. First, the absence in many studies of a significant relationship between the presence of TD and the duration of antipsychotic use may be due to the populations included in the studies, very often patients on long-term antipsychotic treatment. This would tend to obscure any relationship between duration of antipsychotic use and the risk of TD. Indeed, incidence studies with samples of patients in the early years of treatment do show a significant relationship between the duration of antipsychotic use and the development of TD (Kane and Marder, 1993; Kane *et al.*, 1986). This

relationship is even more obvious in older patients receiving antipsychotics for the first time. In those patients, a 1 year incidence of TD of 26% and a 3-year incidence of 60% has been reported repeatedly (Jeste, 2000, 2004).

Second, there are many case reports of tardive syndromes in non-schizophrenic patients who have used antipsychotics for other indications, such as anxiety, personality disorders, hypochondriasis, or behavioral problems (Skidmore *et al.*, 2005). Third, dopamine blocking agents are used in general practice and can induce movement disorders. There is even a black box warning issued by the FDA in 2009 regarding long-term or high-dose use of metoclopramide (dopamine blocking antiemetic) because of the risk of developing TD (Rao and Camilleri, 2010). Fourth, there are case reports of patients in whom tardive dystonia or TD decreased after withdrawal of antipsychotics but increased or reappeared after being re-challenged with these agents. Fifth, it is clear that antipsychotics cause acute extrapyramidal side effects, and in a few cases, acute dystonia developed into persistent dystonia (Skidmore *et al.*, 2005; van Harten *et al.*, 1996a). These considerations have led many clinicians and researchers to conclude that antipsychotics are capable of causing or precipitating persistent dyskinesia or dystonia.

## B. Is Tardive Dyskinesia Still a Problem?

The prevalence of antipsychotic-induced movement disorders in patients on long-term treatment with first-generation antipsychotics (FGAs) is between 50 and 75% (Janno *et al.*, 2004; van Harten, 1998; van Harten *et al.*, 1996b). The introduction of the second-generation antipsychotics (SGAs) led to the expectation that drug-induced movement disorders would disappear. However, the results of 12 long-term studies of SGAs made it clear that SGAs only reduce the risk when compared to FGAs (Correll and Schenk, 2008). Moreover, these studies had several methodological problems such as no equivalent dosage of haloperidol in the control arm, high dropout rates, short study duration, and invalid approaches to measuring movement disorders, all of which limit the validity of the conclusions. Also, these trials were sponsored by pharmaceutical companies, which have a direct interest in the outcome of their product as illustrated by Heres *et al.* (2006).

In contrast, three other, large non-pharmaceutical company-sponsored trials published in the last 5 years did find differences between FGAs and SGAs in the incidence of parkinsonism and akathisia, but almost none in the incidence of TD (Casey, 2006; Jones *et al.*, 2006; Kahn *et al.*, 2008; Lieberman *et al.*, 2005). However, these studies also had methodological problems, such as relatively short follow-up period to detect TD (around 1 year), high dropout rates, and in the Cutlass trial, many patients in the FGA group used sulpiride, which has a lower incidence of extrapyramidal side effects and is classified by some researchers as a SGA.

Furthermore, FGAs are still prescribed and combining FGAs with SGAs is not uncommon in clinical practice (Barbui *et al.*, 2006; Broekema *et al.*, 2007; Ganguly *et al.*, 2004).

Another development of great importance for the absolute number of patients with antipsychotic-induced movement disorders is that SGAs are increasingly used for other indications as SGAs have strong mood stabilizing properties (Citrome *et al.*, 2009). Thus, even if SGAs have a reduced risk, when used in a much larger population (bipolar and other disorders), the total number of drug-induced movement disorders will still be substantial. Therefore, it is not realistic to suggest that drug-induced movement disorders are disappearing.

Recognition and measurement of these disorders and identification of those patients who are particularly at risk of developing TD may pave the way to the development of preventive and treatment strategies, which in turn should increase the quality of life of the patient.

## II. Clinical Features

TD is characterized by involuntary writhing and purposeless, irregular movements that may or may not be continuous. All antipsychotic-induced involuntary movements disappear during sleep, particularly during the deep phase of the sleep. The severity of dyskinesia may change with the patient's level of excitement. In an anxious patient the disorder may be more severe whereas during relaxation it may decrease. Moreover, volitional motor activity may alter the severity, for example, finger tapping or walking may bring out or increase dyskinesia (APA, 1992). This alteration in severity is sometimes wrongly interpreted as evidence that the disorder is of psychogenic origin, particularly if the clinician has made the incorrect assumption that the severity of dyskinetic movements should be constantly the same.

The assessment of the severity of the movement disorder can be based on location, character, amplitude, frequency (i.e., the number of movements occurring in a defined period), and persistence (the proportion of a period during which the movements are apparent). The absence or presence of subjective feelings (often feelings of shame) caused by the involuntary movements are also an indicator of the severity, and for the patient often more important than the "objective" severity based on a rating scale (Emsley *et al.* (2011), Freed, 1982).

The DSM-IV defines the diagnosis of TD by several criteria as formulated in the DSM-IV TR. (APA, 2000). In research, Schooler and Kane (1982) criteria are often used. They define TD as the development of dyskinesia during the use of antipsychotics for a minimum of 3 months or within 3 months (in depot antipsychotics

6 months) after cessation of the antipsychotics; patients should have at least two mild or one moderate score on the Abnormal Involuntary Movement Rating Scale (Guy, 1976; Schooler and Kane, 1982).

There is no specific (laboratory) test to differentiate between schizophrenia-related dyskinesia and drug-induced TD (see differential diagnosis).

TD can be divided into orofacial, limb-truncal, and respiratory dyskinesia and combinations are not uncommon.

### A. Orofacial Dyskinesia

The core sign in orofacial dyskinesia is the bucco-linguo-masticatory triad. This consists of involuntary movements of the tongue, jaw, lips, or face, for example, twisting, curling or protrusion of the tongue, chewing or lateral jaw movements, pursing, sucking, pouting, or puckering of the lips, facial tics, and frequent eye blinking.

The orofacial type is the most common type of TD and accounts for approximately 80% of TD cases (Rapaport *et al.*, 2000). Severe oral dyskinesia may result in dental problems that can progress to ulceration, as well as to muffled or unintelligible speech or impaired eating and swallowing.

Although rare, TD can be accompanied by painful sensations that can become a source of profound distress for the patient (Tschopp *et al.*, 2009). Sometimes the cause of the pain is related to a dental condition in which a dyskinetic tongue brushes over a tooth that is raw (van Harten and Hovestadt, 2006).

However, most of the time oral TD does not cause pain or physical disabilities but if patients are conscious of their dyskinetic movements, social disability is often present. Patients may feel embarrassed, anxious, or depressed when they notice that others observe their dyskinetic movements and the presence of obviously odd movements can lead to stigmatization. Feelings of shame are a common reason for seeking help. One study showed that patients with orofacial dyskinesia are deemed less socially acceptable (Boumans *et al.*, 1994).

### B. Limb-Truncal Dyskinesia

Limb-truncal dyskinesia consists of choreiform purposeless movements of trunk and/or limbs, such as writhing movements of the fingers ("piano-playing fingers") or irregular toe movements, rotation of the wrists, arms, ankles, and legs, head nodding, trunk movements, and pelvic thrusting (Paulsen *et al.*, 1996). Pelvic thrusting can be very socially invalidating and limb and/or trunk dyskinesia may cause gait disturbances and may leave patients vulnerable to falls (Alentorn *et al.*, 2009).

### C. Respiratory Dyskinesia

A fast irregular breathing pattern is the core feature of respiratory dyskinesia. Sometimes other symptoms are present, such as gasping, sighing, and/or grunting, forceful breathing, shortness of breath, and dyspnea (Kruk *et al.*, 1995; Nakamura *et al.*, 1991). Respiratory dyskinesia can even induce dyspnea and cyanosis. The muscles involved are the respiratory musculature or the diaphragm. The involvement of the diaphragm can also induce dyskinetic movements of the belly and is then known as the belly dancer syndrome, which can also be induced by dyskinetic movements of the abdominal muscles.

A clinical rule of thumb is that almost all cases of respiratory dyskinesia are accompanied by orofacial dyskinesia (Yassa and Lal, 1986). In fact, if orofacial dyskinesia is not present, one should re-consider the diagnosis of respiratory dyskinesia.

The patient's lack of awareness of or even denial of dyskinetic movements may be striking and is associated with cognitive impairment and negative symptoms often present in patients with schizophrenia (Chong *et al.*, 2001). However, lack of awareness of TD and lack of insight in schizophrenia are not directly related (Emsley *et al.*, 2011). Patients with schizoaffective, bipolar, or anxiety disorders are more often aware of their movement disorders (Macpherson and Collis, 1992).

## III. Differential Diagnosis

Dyskinesia occurs ideopathically in antipsychotic-naïve patients with psychotic disorders, drug induced (antipsychotics or other drugs), in hereditary diseases, in a systemic or neurological disease, or as a result of psychological stress.

Spontaneous hyperkinetic dyskinesias such as "grimacing" and "irregular movements of tongue and lips" are prevalent in antipsychotic-naïve psychotic patients: Kraepelin and Bleuler described the phenomenon more than 100 years ago (Koning *et al.*, 2010). Also, recent studies indicate that the number of spontaneous movement disorders related to schizophrenia are substantial and increase with age (Fenton, 2000; Koning *et al.*, 2010; McCreadie *et al.*, 2002; Tarbox and Pogue-Geile, 2006).

Schizophrenia stereotypies (purposeless, meaningless actions) and mannerisms (peculiar ways of carrying out normal actions) may be confused with drug-induced dyskinetic movements. Differentiation between dyskinesia related to psychosis and drug-induced dyskinesia can be effected by a careful history regarding the timing of the onset of the dyskinesia in relation to antipsychotic use. However, when the onset of the dyskinesia occurs after the use of antipsychotics, differentiation may be

impossible. In clinical practice the differentiation is often based on the character of the movement. For example, in a patient with schizophrenia, raising the shoulders with each step taken is classified as a stereotypy because the movement differs from movements typically seen in TD. The repeated touching of his hair by a mutistic patient, followed by a grimace and clapping of hands, is classified as mannerism (it may have a magical function) because the movements are far too complex compared to dyskinetic movements. On the other hand, the curling wormlike movements of the tongue developed by a patient on long-term antipsychotic medication are classified as TD because this is a typical dyskinetic movement seen in orofacial TD.

However, another reason for continuous movements in the orofacial region could be ill-fitting dentures or other dental problems. Although these movements are voluntary in contrast to the involuntary movements in TD, patients do not always recognize the voluntary nature of the movements.

TD must also be differentiated from tics. A tic is a stereotyped repetitive, involuntary movement or sound, frequently preceded by premonitory sensations or urges (Shprecher and Kurlan, 2009). These sensations are not present in TD.

Acute drug-induced movement disorders such as akathisia, acute dystonia, or withdrawal syndromes may be confused with TD. Akathisia is characterized by feelings of restlessness and it is this restlessness that forces the patient to move his legs or even his whole body. Acute dystonia is clearly related to the start of or a substantial increase in the dosage of an antipsychotic (or other drugs that block dopamine receptors), or after a sudden withdrawal of an anticholinergic (van Harten *et al.*, 1999). Acute dystonia disappears after an injection of an anticholinergic in contrast to TD, which will persist or even worsen.

Withdrawal emergent dyskinesia is more often seen in children than in adults and develops after a sudden withdrawal of antipsychotics. It is always self-limiting and most of the time it disappears within a few hours or days (Mejia and Jankovic, 2010).

If the dyskinesia is accompanied by dystonic features, Wilson's disease, which can start with dystonic movements, must be ruled out also because it is potentially treatable (Schilsky, 2009; Wiggelinkhuizen *et al.*, 2009).

If the dyskinesia is progressive or is accompanied by other somatic and/or psychiatric signs (such as cognitive impairment, memory, and attention disturbances), diseases such as Huntington's disease, Sydenham's chorea, neuroacanthocytosis, Fahr's syndrome, and Hallervorden-Spatz disease should be considered. Although an increase in the severity of the dyskinesia is a warning to consider other diagnoses, TD or spontaneous dyskinesia related to schizophrenia can be progressive also.

Psychogenic movement disorders can mimic many other movement disorders, most frequently tremors but also such disorders as psychogenic dystonia or myoclonus. Psychogenic movement disorders often start abruptly, after a traumatic

event, in contrast to TD, which emerges gradually (Espay *et al.*, 2009; Miyasaki *et al.*, 2003).

Respiratory dyskinesia is often misdiagnosed as a respiratory disorder or as psychogenic hyperventilation. However, the breathing pattern in hyperventilation is regular in contrast with respiratory dyskinesia, which has an irregular pattern. Furthermore, as mentioned, respiratory dyskinesias almost always go together with orofacial dyskinesia.

Many other drugs can produce hyperkinetic movement disorders that must also be differentiated from TD. We describe a few drugs that may be the cause of dyskinesia or an increase in severity of existing TD. Abuse of amphetamines, cocaine, and other stimulants can cause chorea, dystonia, and stereotyped behavior during use or withdrawal. Anticholinergics and antihistaminic agents have rarely been associated with the onset of orofacial dyskinesias but do increase the severity of dyskinetic movements. Several anticonvulsants can produce dyskinesias resembling TD. Oral contraceptives and the chloroquine-based antimalarial agents can also cause chorea and other dyskinetic symptoms. The use of lithium or tricyclic antidepressants (partly due to their anticholinergic effect) has been associated with aggravating existing TD. These agents can also produce fine, rapid tremors that may be superimposed on TD. Dopamine agonists, and the dopamine precursor levodopa can also provoke hyperkinetic dyskinesias (Casey, 1990).

In clinical practice, the diagnosis of TD is made when a patient has used antipsychotics for at least 3 months (in older patients at least 1 month) or within 3 months after cessation of antipsychotics (in depot antipsychotics 6 months) and dyskinesia is the only symptom. In these patients, the benefits of extensive research into various rare diseases do not weigh against the burden of all the diagnostic procedures for the patient or against the costs.

## IV. Pathophysiology

The pathophysiology of TD is not yet totally understood. We discuss two hypotheses, the dopamine supersensitivity hypothesis, including the related D2 binding affinity theory, because they explain at least some of the characteristics of TD, and the neurotoxicity theory which hypothesis is also linked to movement disorders found in neurology. However, there are other pathophysiological explanations, such as those related to Gamma-aminobutyric acid (GABA) a central role in generating abnormal neuronal activity in subcritical areas or striatal dysfunction. These theories explain dyskinesia by increased activity of the D1-mediated striatonigral/striatopallidal pathway. These striatal neurons are primarily GABAergic but other neuropeptides and neurotransmitters such as dynorphin,

substance P, neurotensin, CCK (cholecystokinin), and NMDA (*N*-methyl D-aspartate, a glutamate receptor), and opioid receptors may also contribute to the development of TD (Hyde *et al.*, 2005; Margolese *et al.*, 2005).

## A. Dopamine Supersensitivity Theory

Receptor supersensitivity can be induced by several biochemical mechanisms such as a change in the interaction of the receptor with G-protein, with guanine diphosphate (GDP), with adenylcyclase, and other messenger systems. These are intracellular mechanisms that trigger the receptor on the cell membrane to act, for example to activate a neuron. When the D2 receptor becomes supersensitive, a shift occurs that causes D2 receptors with a low affinity to a high affinity state. In a high-affinity state, receptors already bind dopamine in lower concentrations; they have become supersensitive. The dopamine supersensitivity theory suggests that long-term dopamine receptor blockage in the striatal area induces supersensitivity which causes dyskinetic symptoms.

This hypothesis explains the inverse relationship between the dosage of the antipsychotics and the severity of the TD: a higher dosage of antipsychotics blocks supersensitive dopamine receptors so that they cannot cause dyskinetic symptoms. Another argument for this theory is that clozapine does not (or very rarely) cause TD nor induce D2 receptor supersensitivity. Furthermore, it is consistent with the fact that early parkinsonism is a risk factor for TD (Tenback *et al.*, 2006).

However, several arguments challenge this hypothesis: (i) supersensitivity of the receptor is already present after a few weeks of antipsychotic use while TD often develops much later, (ii) almost all patients on antipsychotics develop dopamine supersensitivity but not all develop TD, and (iii) after cessation of antipsychotics, the dopamine supersensitivity disappears but TD often persists for years (Hyde *et al.*, 2005).

### 1. *Affinity for D2 Receptor*

The binding affinity theory is closely related to the dopamine supersensitivity theory. Blockage of the D2 receptor is a necessary condition for the antipsychotic efficacy of a drug. There appear, however, to be major differences among antipsychotics in the extent to which the D2 receptor is released, the $K_{off}$ values. The higher the value, the faster the D2 receptor is released. The $K_{off}$ values of clozapine and quetiapine for example are higher, of olanzapin slightly higher, and of risperidone and haloperidol much less than the $K_{off}$ of dopamine. This model explains the differences between antipsychotics regarding the risk of developing acute movement disorders (Kapur and Seeman, 2001). This model also explains the difference in the risk of developing TD. Indeed, a neuroimaging study in humans showed that

dopamine D2 receptor binding is increased after long-term treatment with FGAs and SGAs with high affinity for dopamine D2 receptors (Silvestri *et al.*, 2000).

It is also likely that dopamine supersensitivity is less in antipsychotics that dissociate quickly from the D2 receptor. Indeed, an imaging study in rats suggested that vacuous chewing movements require high D (2) occupancy and that a certain level of D(2) occupancy may be necessary to induce dyskinetic movements (Turrone *et al.*, 2003, 2005). Supporting this theory is the fact that FGAs often induce parkinsonism, which is related to a high and sustained blockage of more than 80% of the D2 receptor, and that parkinsonism is an established risk factor of TD (Mizrahi *et al.*, 2007; Tenback *et al.*, 2009).

Several neuroimaging studies show that for the treatment of psychosis, the most effective range of D2 receptor occupancy is between 60 and 70%, beyond which the likelihood of response diminishes, while risk of D2-related side effects increases (De Haan *et al.*, 2003; de Haan *et al.*, 2004; Remington and Kapur, 2010). These studies also confirmed that over a 24-h interval, D2 occupancy levels can fall well below the recommended threshold without loss of antipsychotic effect. This means that continuous and high levels of D2 occupancy are not always required and it could be that those antipsychotics with the characteristic of dissociating quickly from the receptor carry less risk of TD without the loss of antipsychotic effectiveness (Remington and Kapur, 2010). Moreover, this may explain why clozapine does not induce parkinsonism and very rarely TD and that some SGAs have a reduced risk (Tauscher *et al.*, 2004).

### B. Neurotoxicity

The neurotoxicity hypothesis is related to the free radical theory. A free radical is an atom or molecule that has a single unpaired electron in an outer shell; free radicals are released in the metabolization of dopamine. Dopamine is converted to 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). This process frees hydrogen peroxide, a powerful oxidant that can damage proteins, phospholipids, and other cellular components. Thus oxidation of phospholipids in the membranes of the dopamine-producing cells may induce cell degeneration.

Antipsychotics increase dopamine metabolization and more neurotoxic free radicals are released than cells can handle. This may damage the dopamine receptors, which explains why TD persists even after discontinuation of the antipsychotics (Casey, 2004).

This theory may also explain why TD occurs more rapidly in old age, when the dopamine system is more vulnerable. Genetic data also argue for this hypothesis (see chapter 9 for discussion about genetics). Furthermore, some (but not all, see discussion under Treatment) RCTs (Randomized Controlled Trials) support this hypothesis by showing a beneficial effect on TD with the use of free radical

scavengers such as vitamins E, D, and B6 and melatonin (Lerner *et al.*, 2007; Nelson *et al.*, 2003; Shamir *et al.*, 2001; Soares and McGrath, 2001). It could also explain why free radical scavengers are probably effective in early TD (<1–3 years), when the damage to the receptors may still be reversible (Soares and McGrath, 2001).

Although the ultimate model for TD has not yet been formulated, it is plausible that several of these vulnerabilities and mechanisms act together to produce TD (Margolese *et al.*, 2005).

## V. Tardive Dyskinesia Treatments

More than 500 RCTs evaluating over 90 different interventions did not generate enough evidence to make a guideline on "how to prevent or treat TD." This conclusion is based on the extensive reports by the Cochrane Schizophrenia Group about RCTs concerning the treatment of TD (Soares-Weiser and Fernandez, 2007). Interpretation of these RCTs are hampered by the poor methodology used in most of these trials, in particular in regard to small sample size (thereby reducing the power of the study considerably) and short duration of follow-up (most studies are shorter than 8 weeks). TD develops over a long period and for many treatments, it takes considerable time to evaluate the effect of treatment. Furthermore, baseline values are often based on a single measurement while TD symptoms fluctuate over time. It may be preferable to measure the TD at two consecutive 3-months intervals. If TD is diagnosed in both measurements such patients can then be categorized as having persistent TD. Any improvements found would then have more clinical value. The RCTs that compared a treatment with a placebo included trials with anticholinergics (actually the withdrawal of), benzodiazepines, calcium channel blockers, cholinergics, GABAergic compounds, antipsychotic medication (including dose reduction and cessation of the antipsychotic), catecholaminergics, and vitamin E.

We discuss the results of interventions that are regularly used in clinical practice based on the Cochrane reports. For further details, see the Cochrane Library (Soares-Weiser and Fernandez, 2007). Furthermore, we discuss three promising treatments, tetrabenazine, botulinum toxin, and deep brain stimulation. Thereafter, we will suggest prevention and treatment recommendations.

### A. Cessation or Reduction of the Antipsychotic Dosage

Discontinuation of antipsychotics must be weighed against the risk of a psychotic relapse. However, many patients receive antipsychotics for indications

other than a psychotic disorder, such as borderline personality disorder, behavioral problems in elderly or mentally disabled patients, anxiety, automutilation, or autism spectrum disorder, and sometimes as an antiemetic (metoclopramide). In the case of all of these non-psychotic indications, cessation should be considered. This is even more indicated in older patients who are highly vulnerable for developing TD. However, there are no relevant TD RCTs that compare discontinuation versus maintenance treatment. Most discontinuation studies are not randomized, hindering an objective interpretation. In these studies, discontinuation or reduction was favorable over the long-term in 30–50% of the patients (Jeste *et al.*, 1988). However, some of these studies showed that after discontinuation of the antipsychotics there was an increase in the severity of TD and dysphoria or a psychotic relapse, resulting in the early removal of subjects from the study. Two small trials showed that a reduction of the antipsychotic dose resulted in more than a 50% improvement in TD compared to no reduction (Soares-Weiser and Rathbone, 2006).

### B. Switching to Clozapine

Clozapine does not or very rarely induce TD. In the few case reports suggesting that clozapine induces TD most of the patients had a history of long-term use of FGAs. In such cases switching to clozapine may reveal suppressed TD (Bruscas *et al.*, 2007; Duggal and Mendhekar, 2007; Ertugrul and Demir, 2005; Li *et al.*, 2009; Raguraman and Vijaysagar, 2007).

In one trial, clozapine was compared with haloperidol for the treatment of patients with TD. Clozapine produced significantly greater motor symptom benefit after 12 months of treatment than did haloperidol. Moreover, the dyskinesia rebound, which occurred equally in both drug groups at the beginning of the study, was sustained in the haloperidol group but disappeared in the patients treated with clozapine (Tamminga *et al.*, 1994). These data suggest that dyskinetic symptoms decrease, along with dopaminergic hypersensitivity, with long-term clozapine treatment (Lieberman, 2007; Lieberman *et al.*, 1991).

There are several open trials and case series or case reports suggesting a beneficial effect of clozapine on existing TD. It seems that clozapine is especially beneficial in those patients in which TD is combined with tardive dystonia (Louza and Bassitt, 2005; van Harten *et al.*, 1996a).

### C. Switching to Another SGA Than Clozapine

According to a systematic review, the annualized incidence of TD was 3.9% for people taking SGAs and 5.5% for FGAs (Correll and Schenk, 2008). This

suggests that switching patients with TD to a SGA other than clozapine may be beneficial. However, only a few studies have addressed this issue. In a 12-month, randomized, investigator-blinded study, the efficacy of quetiapine ($N = 22$) and haloperidol ($N = 23$) was compared in patients with schizophrenia or schizoaffective disorder and established TD. Compared to the haloperidol group, the quetiapine group showed significantly greater improvements (Emsley *et al.*, 2004).

A randomized 24-week single blind trial ($N = 60$) with patients with schizophrenia on treatment with FGAs and with TD compared the results of a switch to risperidone versus olanzapine. Both groups showed a reduction in TD (Chan *et al.*, 2010).

In a large observational study, the TD in those patients switched to an SGA showed less persistence than those switched to a FGA (Tenback *et al.*, 2010)

### D. Treatment With Cholinergic Medication

TD may include a central cholinergic deficiency. Therefore, cholinergic drugs (arecoline, choline, deanol, lecithin, meclofenoxate, physostigmine, RS 86, tacrine, metoxytacrine, galantamine, ipidacrine, donepezil, rivastigmine, eptastigmine, metrifonate, xanomeline, cevimeline) have been used to treat TD. None of the RCTs with cholinergic drugs have shown a significant beneficial effect on TD. However, the sample size of most studies was small (5–20) and the new cholinergic Alzheimer drugs have not been tested yet (Tammenmaa *et al.*, 2004).

### E. Treatment with Benzodiazepines

Because of the sedative, anxiolytic, anticonvulsant, and particularly the muscle relaxant effects of benzodiazepine, these drugs have been used as an adjunct to the antipsychotic treatment to treat TD. Three trials using benzodiazepines (total $N = 56$) did not find an important clinical improvement. Only one small study reports some preliminary evidence that benzodiazepines may have some effect in antipsychotic-induced TD (Bhoopathi and Soares-Weiser, 2006).

### F. Withdrawal of Anticholinergic Drugs

A substantial number of psychotic patients using antipsychotics also receive anticholinergic drugs to reduce some of the acute drug-induced movement disorders such as akathisia and parkinsonism. It has been observed that withdrawal of anticholinergic drugs reduced the severity of TD. However, the Cochrane review concluded that there were insufficient data to make a conclusion about withdrawal

or adding anticholinergics for patients with TD (Rathbone and Soares-Weiser, 2006). Furthermore, some patients experience a worsening of akathisia and/or parkinsonism after withdrawal and this often leads to more discomfort than the discomfort related to TD

## G. Adding Antioxidants

As discussed above, the neurotoxic hypothesis of TD is related to free radicals generated by the metabolization of antipsychotics. In line with this theory, antioxidants may minimize the neurotoxic effect of these free radicals. Most studies were done with vitamin E and several small studies show a decrease in the severity of TD, particularly in those in whom the onset of the TD occurred in the preceding 5 years (Soares and McGrath, 2001). Other antioxidants such as melatonin and vitamin B6 were also effective in small RCTs (Lerner *et al.*, 2001, 2007; Shamir *et al.*, 2001).

One large long-term RCT of vitamin E versus placebo found no evidence for the efficacy of vitamin E in the treatment of TD. However, the mean duration of TD of the population was long (over 3 years) and this could have induced a bias because after several years, the damage to the dopamine receptors may be irreversible (Adler *et al.*, 1999).

Despite these results, the Cochrane concluded that small trials with uncertain quality of randomization indicate that vitamin E protects against deterioration of TD but that there is no evidence that vitamin E improves the symptoms of TD (Soares-Weiser and Fernandez, 2007).

A clinically important question that has not been studied yet is the preventive properties of vitamin E. An RCT of vitamin E versus placebo in patients who are starting with antipsychotics is indicated, in particular for older patients, who are very vulnerable for TD.

## H. Potentially Promising Treatments

### *1. Tetrabenazine*

Tetrabenazine is a monoamine depletor and a dopamine receptor blocker used to treat several hyperkinetic movement disorders. It has been registered to treat dyskinetic movements in Huntington's chorea (Kenney and Jankovic, 2006). One small study ($N = 20$) without a control group but with blind assessment by randomized videotape protocol showed significant improvement (Ondo *et al.*, 1999). Furthermore, several case reports and case series showed improvement, often within a few weeks, in patients with severe forms of TD. However, the

evidence is not sufficient and furthermore 10–15% of the patients receiving tetrabenazine developed depressive feelings, which were even more severe in patients with a history of depression (Jankovic and Orman, 1988; Kenney and Jankovic, 2006; Kenney *et al.*, 2006).

### 2. *Botulinum Toxin*

Botulinum toxin is established as an effective therapy for focal dystonic disorders such as blepharospasm and torticollis (Bouchard *et al.*, 2010; Ellison and Wandres, 1992; Gill and Kraft, 2010). Botulinum toxin may also be useful in severe TD, especially when accompanied by dystonic features (a combination of TD and tardive dystonia). Several case reports show the effectiveness in orofacial dyskinesia complicated by tongue protrusion, which can be socially disabling (Charles *et al.*, 1997; Hennings *et al.*, 2008; van Harten and Hovestadt, 2006).

A small single blind (raters were blind) study ($N = 12$) showed a non-significant reduction in the severity of orofacial dyskinesia (Slotema *et al.*, 2008).

### 3. *Deep Brain Stimulation*

Deep brain stimulation is a well-known treatment for Parkinson's disease (Bronstein *et al.*, 2011). In patients with severe forms of TD combined with dystonia, several case reports have shown remarkable improvement (Cohen *et al.*, 2007; Damier *et al.*, 2007; Eltahawy *et al.*, 2004; Koller *et al.*, 2001; Schrader *et al.*, 2004; Trottenberg *et al.*, 2005; Zhang *et al.*, 2006). One small study ($N = 10$) of the French Stimulation for TD Study Group (STARDYS) showed that patients with severe TD unresponsive to previous treatment could benefit from bilateral deep brain stimulation of the globus pallidus. After 6 months, a double-blind evaluation in the presence and absence of stimulation showed that all 10 patients had improved (mean improvement, 61%; range, 44–75%). There were no marked changes in the patients' psychiatric status (Kefalopoulou *et al.*, 2009). Although a larger and longer trial is needed, this study suggests that bilateral globus pallidus stimulation offers a new treatment option for disabling TD.

## VI. Prevention and Treatment of Tardive Dyskinesia in Clinical Practice

Given the lack of firm evidence for the treatment of best practices, it is not possible to formulate exact clinical guidelines. However, in daily practice clinicians are asked to treat these patients. Therefore, we will try with the aid of the literature and clinical experience to present practical advice for preventive and treatment strategies.

## A. Prevention of TD

### 1. *Communication With the Patient*

Inform the patient or the family about the risk of TD as a result of prolonged use of antipsychotics. This is important from legal and ethical points of view, necessary for obtaining informed consent to treatment, and also to alert the patient (or family) to this side effect. Some doctors fear that this will provoke patient non-compliance but this is not our experience and one study found that giving information about TD did not have a negative impact on medication compliance (Chaplin and Kent, 1998; Chaplin and Timehin, 2002). On the other hand, uninformed patients or family can be very distressed or annoyed when TD "unexpectedly" develops. It is often not possible to inform a patient during an acute psychotic episode but information can be given when the patient has stabilized.

### 2. *Regular Systematic Screening for TD*

The www.psychiatrynet.eu section on movement disorders provides a guideline for systematic screening (www.psychiatrynet.eu/druginduced.pdf). Such an exam consists of observing the patient's body parts, extremities, tongue, and mouth under resting conditions as well as in response to a motor task that acts as a stressor, for example touching the thumb and fingers together in sequential fashion or walking. These motor tasks are provocative tests and help to judge the TD, to uncover covert TD, and to check whether those activities provoke involuntary movements in other regions of the body. Such a screening is recommended for patients on long-term antipsychotic treatment every 6 months (APA, 1992; Lieberman, 2007). Special attention should be given to the breathing pattern as respiratory dyskinesia is often missed or misdiagnosed.

### 3. *Re-evaluation of the Necessity and Dosage of the Antipsychotic*

When antipsychotics are used for non-psychotic indications, alternative treatment is often available and discontinuation possible. In psychotic disorders, discontinuation usually significantly increases the risk of a psychotic relapse. The alternative, intermittent antipsychotic treatment probably increases the risk for TD (Gaebel, 1994; Goldman and Luchins, 1984; van Harten *et al.*, 1998). However, the dosage of the antipsychotic can often be reduced over time.

### 4. *Awareness of the Risk Factors*

The clinical significance of a risk factor depends on its prevalence and the possibility of mitigating it (how well can the consequences be treated) Chapter 9 discusses this aspect thoroughly.

5. *Change of Medication*

   Start with or switch to an atypical antipsychotic, as discussed above

## B. Treatment of TD

If patients have developed TD, the main points are to determine whether discontinuation of the antipsychotic is possible and how much suffering the TD causes the patient. As described above, some patients are not aware of their orofacial or other movement disorders and do not complain about it. In such cases, especially when the TD is not severe and the risk of a psychotic relapse is considerable, one should be cautious about interfering. However, the family may be concerned about the movement disorders, especially when the TD is severe or disabling. The patient's preferences about treating the TD must be also be taken into account.

If discontinuing the antipsychotics is possible, clinicians should be aware that tardive syndromes may worsen, at least initially. However, regularly after 6–12 weeks, the severity is back to the severity level before discontinuation and several follow-up studies show that, in the long run, discontinuation reduces the severity of TD, especially when the duration of TD is short and patients are younger than 50–60 years (Fernandez and Friedman, 2003; Jeste and Wyatt, 1982).

Main treatment options if cessation of antipsychotics is not possible are as follows:

1. *Lowering the dosage*: This is the usual recommended step. Although the effectiveness is not established, lowering the dosage is often also beneficial for other side effects (APA, 1992; Fernandez and Friedman, 2003; Soares-Weiser and Fernandez, 2007).
2. *Switching to clozapine*: Based on follow-up trials and also in our experience, switching to clozapine is often more effective than lowering the dosage, especially when TD is combined with tardive dystonia. Sometimes the effect is already perceptible within a few weeks but sometimes it takes more time (3–6 months) to evaluate the result. It has been suggested that the improvement is not only due to the passage of time in the absence of the antipsychotic that caused the TD but also because of therapeutic effect of clozapine (Dalack *et al.*, 1998; Lieberman *et al.*, 1991; Tamminga *et al.*, 1994, van Harten *et al.*, 1996a).
3. *Switching to another SGA*: See discussion above.
4. *Adding an antidyskinetic agent*: If the preceding steps are not effective (enough) and the TD and or tardive dystonia is severe or patients complain about it, our movement disorder center (Amersfoort, the Netherlands) regularly advises botulinum toxin or tetrabenazine. Botulinum toxin is indicated in

focal dystonic symptoms and in tongue protrusion (botulinum toxin in the genioglossus) or can also be given for orofacial dyskinesia (Slotema *et al.*, 2008; van Harten and Hovestadt, 2006). Tetrabenazine is indicated for dyskinetic or dystonic symptoms present in different body parts (Kenney and Jankovic, 2006; Ondo *et al.*, 1999).

5. *Referral for Deep Brain Stimulation*: Referral to a neurosurgical center for deep brain stimulation is indicated for severe forms that do not react to the preceding measures (see above) (Damier *et al.*, 2007).
6. *Increasing the dosage of the antipsychotics*: The mechanism, as described above, is that an increase in the antipsychotic dosage increases dopamine receptor blockage and that may decrease the severity of dyskinetic and dystonic symptoms. However, this step should only be used in extreme situations such as severe generalized TD or dystonia that is either painful or causes muscle damage (confirmed by elevated serum Creatine phosphokinase (CPK)) which can be life threatening. Increasing the antipsychotic dosage can also be helpful when all other treatments have failed. Beware that this step may also be limited by the increase of other side effects (Skidmore *et al.*, 2005).

## VII. Conclusion

TD is a severe side effect of dopamine blocking agents and although SGAs present less risks of TD, it remains a clinical issue. Prevention may be more important than treatment because of a lack of clearly effective treatment options.

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# EPIDEMIOLOGY AND RISK FACTORS FOR (TARDIVE) DYSKINESIA

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Dyskinesia can develop in patients with schizophrenia in the course of the disease with or without the use of antipsychotics.In patients with psychiatric disorders other than schizophrenia Tardive Dyskinesia (TD) can develop in patients treated with antipsychotics or other drugs with dopamine D2 blocking properties. Spontaneous Dyskinesia in antipsychotic naive patients with schizophrenia ranges from 4 to 40%, depending on the age and duration of the illness. Moreover, siblings of patients with schizophrenia have higher prevalence rates of dyskinesia than matched controls. Incidence rates of TD due to dopamine blocking properties vary due to the sample population and the affinity for the dopamine blocker to the D2 dopamine receptor. Once developed, TD seems very persistent, the course of TD might be mediated by the affinity for the dopamine D2 receptor. Risk factors for TD in the literature are numerous, in this chapter only replicated and risk factors from longitudinal studies will be reported limiting the amount of

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00009-2





0074-7742/10 $35.00

risk factors. Furthermore only meta-analyses on genetic factors related to TD will be discussed due to inconsistency of genetic effects because of sample heterogeneity, small effects of multiple genes, (epi)genetic interactions, pleiotropy and small sample size. Finally the concept "Tardive Dyskinesia" will be discussed and the influence hereof on the above mentioned factors.

## I. Introduction

Tardive dyskinesia (TD) is a movement disorder characterized by involuntary, choreoathetoid movements in the orofacial region, limbs, trunk and/or respiratory system. Most clinicians in psychiatry view TD as an antipsychotic related side effect and unrelated to the underlying disease for which the antipsychotics are prescribed, yet relatively little is known about the etiology of TD, the clinical consequences of its diagnosis, and treatment.

### A. Tardive Dyskinesia

Movement disorders as side effects of medications are reported since the introduction of antipsychotic medication. The term "Tardive Dyskinesia" was first introduced in 1964 (Faurbye *et al.*, 1964). The first reports of tardive dystonia as a side effect of antipsychotic drugs date back to 1982 (Burke *et al.*, 1982). The current DSM IV describes both TD and acute dystonia as a side effect of antipsychotics. The term tardive dystonia, however, is not mentioned as a solitary entity in the psychiatric classification of side effects of antipsychotics and is currently in the shared domain of TD (DSM-IV, 2000).

### B. Spontaneous Dyskinesia Versus Tardive Dyskinesia

There are many types of dyskinesia associated with neurological diseases like Morbus Huntington, Sydenham's chorea, Hyperthyroidism, Morbus Wilson, and last but not least spontaneous dyskinesia in the elderly.

After the introduction of antipsychotics in 1952, dyskinesia symptoms became associated with antipsychotic treatment. However, the notion that Dyskinesia in psychiatry is due to antipsychotic treatment needs refinement because involuntary hyper- and hypokinetic movements have already been documented in patients with schizophrenia long before the introduction of antipsychotic medication (Kraepelin, 1919). The patients with these movement disorders were diagnosed with "Dementia Preacox," now known as schizophrenia. Thus, a refinement could be, dyskinesia can develop in patients with schizophrenia in the course of the

disease with or without the use of antipsychotics, in patients with psychiatric disorders other than schizophrenia TD can develop in patients treated with antipsychotics. This is still not fully correct because although TD is mainly attributed to the treatment with antipsychotics, other medications like SSRIs, tricyclic antidepressants, or anti-emetics in rare cases also cause TD.

The notion that in psychiatry only antipsychotic naive patients with schizophrenia have dyskinesia is based on the fact that "spontaneous dyskinesia" is only reported in schizophrenia (Pappa and Dazzan, 2009) or in schizotypical disorder (Mittal *et al.*, 2008). Patients with schizotypical personality disorder, however, are at a risk of developing schizophrenia (Dragt *et al.*, 2011). Finally, the lack of literature about spontaneous dyskinesia in other psychiatric disorders does not mean it is not existent. It remains a caveat in the current knowledge.

Thus when discussing the epidemiology of TD, spontaneous dyskinesia in schizophrenia and TD possibly including other tardive forms of movement disorder like dystonia and akathisia in mixed populations in psychiatry will be discussed.

## II. Spontaneous Dyskinesia in Psychiatry

### A. Prevalence Rates of Spontaneous Dyskinesia in Patients with Schizophrenia

Tardive means slow or belated onset. Therefore in schizophrenia the naming Tardive Dyskinesia poses a semantic challenge. Antipsychotic naive patients with schizophrenia develop or already display more dyskinesia than normal matched control subjects (Koning *et al.*, 2008; Pappa and Dazzan, 2009), which concurs with the early literature in dementia praecox (Kraepelin, 1919).

A review of the literature suggested spontaneous dyskinesia prevalence rates of approximately 4% in first-episode schizophrenic patients, 12% for patients ill several years but below 30 years of age, 25% for those aged between 30 and 50 years, and 40% for those aged 60 years or older (Fenton, 2000). However, instrumental measurements in first episode antipsychotic naive patients (mean age 23.6 SD (6.2)) with schizophrenia suggest higher prevalence rates of 13–20% for dyskinesia (Cortese *et al.*, 2005).

### B. Prevalence Rates of Spontaneous Dyskinesia in Siblings of Patients with Schizophrenia

The instrumental measurements are more sensitive than clinical movement rating scales like for example, the AIMS, is illustrated in research where both

traditional measurement scales and instrumental measurements were used appraising movement disorders in siblings of patients diagnosed with schizophrenia. Based on the clinical assessment of movement disorders with the AIMS in a small study comparing movement disorders in siblings of patients with schizophrenia and matched controls 7% of siblings and 3% controls met the clinical definition of dyskinesia versus 21% in siblings and 5% controls when measured mechanically (Koning *et al.*, 2011).

## C. Tardive Dyskinesia Associated with Antipsychotics

There are meta-analyses reporting on incidence and prevalence rates of TD. These meta-analyses take age and type of antipsychotic into account by reporting TD in different age strata and per type of antipsychotic. Antipsychotics differ in their propensity to induce TD. Antipsychotics are divided into first- (FGA) and second-generation antipsychotics (SGA). The FGA characterized by a higher affinity to the dopamine receptor than the SGA (Kapur and Seeman, 2001) and are generally more inclined to induce TD (Correll and Schenk, 2008; Miller *et al.*, 2007).

### *1. Incidence rates of TD in patients taking antipsychotics*

Current meta-analyses report on mixed psychiatric classified populations. Combined for all age strata and psychiatric diagnoses, incidence rates for TD are 5.5% for First Generation Antipsychotics (FGA) and 4.0% annually for Second Generation Antipsychotics (SGA) (Correll *et al.*, 2009).

When reporting on incidence rates of TD for adult patients with schizophrenia incidence rates are 3.0% (SGA) and 7.7% (FGA) respectively (Correll *et al.*, 2009).

### *2. First episode schizophrenia and antipsychotics*

Incidence rates of dyskinesia in first episode patients treated with either haloperidol (FGA) or risperidone (SGA) were 2.33% (5 of 215, 95% confidence interval [CI] = 0.31–4.34) and 0.87% (2 of 229, 95%CI = 0.00–2.08), respectively. Annualized rates were 1.87 and 0.72% respectively using low equivalent doses of both FGA and SGA and a median treatment length of 206 days (Schooler *et al.*, 2005).

### *3. Chronically treated patients*

In a cohort with chronic patients from African descent using antipsychotics, with a mean duration of antipsychotic treatment of 18 years, incidence rates of TD and tardive dystonia were 10.2% (95%CI = 7.7–13.5) and 0.7% (95%CI = 0.4–1.5) respectively (van Harten *et al.*, 2006). This is high because when analyzing incidence

rates, patients with the disorder of interest are removed from the analysis because they already display the disorder, thereby removing the person who already developed the disorder and were probably most at risk. The fact that the incidence is still high indicates that patients remain at risk even after years of treatment with antipsychotics and is of serious clinical concern.

*4. Mixed populations*

In children and adolescent with diagnoses of disruptive behavior disorders ($n$ = 688, 87.9%), bipolar disorder ($n$ = 52, 6.6%), schizophrenia/schizoaffective disorder ($n$ = 26, 3.3%), and autism spectrum disorders ($n$ = 17, 2.2%), the crude annualized TD incidence rates were 0.38%; 95%CI (0.08, 1.11) and 0.42%; 95% CI (0.09, 1.24) for FGA and SGA, respectively (Correll and Kane, 2007). Adjusted TD incidence rates of adult patients with bipolar disorder in a large naturalistic cohort were presented; FGA HR 2.64 95%CI (1.94, 3.60) and SGA HR 2.18 95% CI (1.20, 3.97) respectively versus no antipsychotic (van Rossum *et al.*, 2009).

In the elderly TD incidence rates are reported of respectively 5.2% with second-generation antipsychotics versus 5.2% with first-generation antipsychotics ($P$ = 0.865). This last incidence rate is based almost exclusively on one retrospective cohort study (Correll and Schenk, 2008) and is not in line with previous reported incidence rates in the elderly. A prospective study reporting on 266 patients using high and low potency first generation antipsychotics reports a cumulative incidence of TD of 26, 52, and 60% after 1, 2, and 3 years, respectively (Jeste *et al.*, 1995). The same author reported on a trial with low doses of SGA and reported a cumulative 1 year incidence of TD of 2.6% (Jeste *et al.*, 2000).

## D. Prevalence Rates

The following prevalence rates for TD were presented in a meta-analysis in a psychiatric diagnostically mixed population. Prevalence rates were 32.4, 13.1, and 15.6% for FGAs, SGAs, and antipsychotic naive patients with schizophrenia respectively (Correll *et al.*, 2009).

## E. Course of Tardive Dyskinesia

In the era of the FGA, several studies reported on the course of TD. Some studies report improvement of TD over time (Fernandez *et al.*, 2001) while others report a more or less steady state (Bergen *et al.*, 1989) or worsening of symptoms (Barnes *et al.*, 1983). A study in the era of the SGA evaluated the course of TD while changing class of antipsychotic. A differential effect for persistence of TD symptoms and class of antipsychotic was reported in this study, where patients with TD

at baseline who were receiving second-generation antipsychotics were less likely than patients receiving first-generation antipsychotics to have TD symptoms at 6 months (43.6% vs. 60.8%, odds ratio (OR) = 0.50, 95%CI = 0.30–0.85) (Tenback *et al.*, 2005). A randomized single blind study found similar results for the differential effects on the persistence of TD in an albeit small study (Emsley *et al.*, 2004). This suggests an effect of the affinity to the Dopamine D2 receptor on the presentation of TD.

### F. Persistence Rates of Dyskinesia

In the first antipsychotic era, incidence rates of TD were estimated to be 5% (Kane *et al.*, 1984) with persistence rates of 32, 57, and 68% after 5, 15, and 25 year respectively (Morgenstern and Glazer, 1993).

In the second antipsychotic era, incidence rates of TD are lower because of the lower propensity of SGA to induce TD (Correll *et al.*, 2004). In a study where patients were treated with SGA, the incidence rate was 0.77% (95%CI: 0.50, 1.19) with a subsequent persistence rate of 82% (95%CI: 59, 94%) (Tenback *et al.*, 2010).

### G. Non-Therapeutic Risk Factors for TD

There are numerous articles reporting on risk factor for TD. Many of them are retrospective or cross-sectional and conducted in patients using antipsychotic with all kinds of psychiatric diagnoses. However, when considering risk factors basic epidemiological concepts have to be adhered to including: (i) obtaining the exposure of interest prospectively in a sample free of the outcome of interest at baseline, (ii) defining and sampling the population, and (iii) defining the outcome clearly and measuring it validly and completely.

A meta-analysis looking at non-therapeutic risk factors found only eight studies satisfying the basic epidemiological rules for risk factor for TD in schizophrenia and reported on 25 different single estimate risk factors. Of the 25 risk factors, only six concerned replicated estimates suitable for meta-analysis. Of these six, two risk factors (non-white ethnic group and early EPS) predicted TD in both the fixed and random effects model in schizophrenia, while age predicted TD in the fixed but not in a random model. Female sex, dose, and akathisia did not predict TD (Tenback *et al.*, 2006b).

Other non-replicated risk factors derived from longitudinal studies are tardive dystonia (van Harten *et al.*, 2006), treatment non-responders (Chakos *et al.*, 1996), worse premorbid functioning (Strous *et al.*, 2004), motor sequencing factor from the Neurological Evaluation Scale (NES) (Emsley *et al.*, 2005), percentage change in negative symptoms (Oosthuizen *et al.*, 2003), poor prognosis and long treatment

duration (Chouinard *et al.*, 1988), worsening of psychosis (Tenback *et al.*, 2007), prolactine-related sexual dysfunction (Tenback *et al.*, 2006b).

## H. Risk Factors Associated with Pharmacogenetics

In the light of risk for TD, particular interest in the literature is shown for single nucleotide polymorphisms (SNPs) in genes related to schizophrenia, and the pathophysiology of TD including prolonged blockade of post-synaptic dopamine receptors, post-synaptic dopamine hypersensitivity, damage to striatal GABA interneurons, and damage of striatal cholinergic interneurons through the mechanism of oxidative stress (Margolese *et al.*, 2005).

However, studies on genetic factors related to TD are often inconsistent owing to sample heterogeneity, small effects of multiple genes, (epi)genetic interactions, pleiotropy and small sample size (Abdolmaleky *et al.*, 2005). Because of these limitations, only meta-analyses on the subject will be discussed.

## I. Dose-Related SNPs

Antipsychotics are metabolized by the cytochrome P450 (CYP) isoenzymes, especially by CYP2D6, CYP1A2, and CYP3A4. As the genes coding for CYP2D6 and CYP1A2 are genetically polymorphic resulting in the alteration of the pharmacokinetics of antipsychotics, they have been considered as candidate genes for the development of TD (Ellingrod *et al.*, 2002; Patsopoulos *et al.*, 2005). Although CYP3A4 metabolizes more than 50% of all known drugs and the gene is highly inducible, the wide inter-individual variation cannot be explained by functional polymorphisms, as they are few in number (Tiwari *et al.*, 2005a, 2005b).

CYP2D6 is the most notorious and metabolizes about 25% of all drugs. The multi-allelic system in CYP2D6 gene is responsible for the phenotypic variety consisting of ultrarapid, extensive, intermediate, and slow metabolizers for CYP2D6 metabolizers (UM, EM, IM, and UM, respectively) (Patsopoulos *et al.*, 2005; Zhou, 2009a). Approximately, 18% of antipsychotics are substrates of CYP1A2 enzymes, 40% of CYP2D6, and 23% of CYP3A4. About 10–20% of Western populations are defective in genes of the CYP superfamily. Only 26% of Southern Europeans are pure extensive metabolizers for the trigenic cluster integrated by the CYP2D6 + CYP2C19 + CYP2C9 genes (Cacabelos and Martinez-Bouza, 2010). Recommendations on therapeutic drug monitoring have been made for the following antipsychotics: perphenazine, zuclopenthixol, risperidone, and haloperidol (Zhou, 2009b). Although antipsychotic dose could be deemed a proxy measure the rate of metabolism of an antipsychotic, dose of antipsychotics was not considered to be a risk factor in a meta-analysis evaluating risk factors for TD in schizophrenia (Tenback *et al.*, 2009).

*1. Catechol-O-methyltransferase (COMT)*

Catechol-*O*-methyltransferase is an enzyme catalyzing the degradation of catecholamines thus diminishing the dopamine load. Catechol-*O*-methyltransferase contains an SNP located in exon 4, a G–A substitution at codon 158 changing valine (Val) to methionine (Met), causing a missense mutation resulting in a lower metabolic activity and lower stability form Met of the COMT enzyme (Bakker *et al.*, 2008). A meta-analysis for an association between TD and $COMT^{val158met}$ (rs4680) in mixed population using Val–Val homozygotes as reference reported a protective effect with an OR of 0.63 (95%CI: 0.46–0.86, $P = 0.004$) for Val–Met genotype and 0.66 (95%CI: 0.49–0.88, $P = 0.005$) for Met carriers using fixed effect models (Bakker *et al.*, 2008). Similar results were reported in a sex-stratified meta-analysis of COMT and TD in a population with schizophrenia or a schizo-affective disorder, and reported an association between ValVal genotype and TD in females (OR(ValVal) = 1.63, 95%CI: 1.09–2.45; $P = 0.019$) but not in males (Zai *et al.*, 2010b). The significant higher OR for the ValVal genotype and TD seems related to a higher dopaminergic load.

*2. CYP1A2*

CYP1A2 is involved in the metabolism of 18% of all neuroleptics drugs (Cacabelos and Martinez-Bouza, 2010).

In the analyses of CYP1A2*1F (rs762551), no significant pooled OR was evident for allelic or genotypic comparisons of this polymorphism (Bakker *et al.*, 2008). However, another meta-analysis reported a positive association (Thelma *et al.*, 2008)

*3. CYP2D6 and TD*

A meta-analysis performed by Patsopoulos *et al.* (2005) showed an OR of 1.43 [95%CI 1.06–1.93] of increase in TD for the single comparison group of deficient alleles (*3, *4, *5) together. Positive associations between CYP2D6*10 and TD have been established (Thelma *et al.*, 2008)

## J. SNPs Possibly Related to the Pathophysiology of TD

*1. Dopamine 2 receptor (DRD2)*

A number of allelic association studies focused on polymorphisms in genes coding for the dopamine 2 receptor (DRD2). Meta-analyses studied the association between DRD2 and clinical antipsychotic response, and dyskinesia (Bakker *et al.*, 2008; Zhang *et al.*, 2010).

A meta-analysis for an association between SNPs in DRD2 and TD reported a risk-increasing effect for the A2 variant in *Taq1*A (rs1800497) using the A1 variant as reference category (OR = 1.30, 95%CI: 1.03–1.65, $P = 0.026$) and A2–A2 homozygotes using A1–A1 as reference category (OR = 1.80, 95%CI: 1.03–3.15, $P = 0.037$) (Bakker *et al.*, 2008).

*2. Manganese superoxide dismutase (MnSOD)*

Manganese superoxide dismutase (MnSOD) is one of the enzymes related to the oxidative stress theory of TD (Hori *et al.*, 2000). A meta-analysis for an association between TD and MnSOD Ala–9Val (rs4880) using Ala–Ala homozygotes as reference reported a protective effect for Ala–Val (OR = 0.37, 95%CI: 0.17–0.79, $P = 0.009$) and for Val carriers (OR = 0.49, 95%CI: 0.24–1.00, $P = 0.047$) (Bakker *et al.*, 2008). Another meta-analysis did not report this association (Zai *et al.*, 2010a).

*3. DRD3*

The Dopamine 3 receptor (DRD3) showed evidence for an association between Ser9Gly (rs6280) and TD in two early meta-analyses (Lerer *et al.* 105–119; Bakker, van Harten, and van 185–192), however, no or little evidence in a recent meta-analysis (Tsai *et al.* 57–66).

## III. Discussion

TD in schizophrenia is a severe symptom or syndrome with a negative impact on disease outcome. Patients with persistent TD are less likely to experience psychiatric symptom remission, and have more severe extrapyramidal side effects, and lower levels of quality of life and functioning, lower productivity, and fewer activities (Ascher-Svanum *et al.*, 2008). In general SGA pose a lower risk of TD than FGA, however once TD emerges while using FGA is seems to persist.

This chapter aims to appraise incidence, course, and patient-related and medication-related risk factors for TD. However, in order to discuss risk, a frame of reference with regard the definition of TD has to be established. Therefore, the term "Tardive Dyskinesia" needs delimiting in order to discuss the epidemiology of TD.

Involuntary movements such as dystonia, myoclonus, tics, tremor, and akathisia could be considered manifestations of dysregulation of the dopamine system caused by direct blockade of the dopamine receptor, structural changes of the dopamine system (e.g., sensitization of dopamine receptor), or interaction with other neurotransmitter systems, Previously, the term dyskinesia, Greek for

"difficulty of movement," covered all these movements. Tardive comes from French word tardif meaning tardy or late, referring to the fact that signs of dyskinesia appear late in the course of drug treatment.

In general, tardive dystonia is seen as a variation of TD (Burke and Kang, 1988) which aligns with the fact that most scales measuring TD make no distinction between TD and dystonia (Chouinard and Margolese, 2005; Guy, 1976a).

However, when looking at dyskinesia, dystonia, myoclonus, tremor, and akathisia, there are distinct phenomenological differences, which raise the question if this is indeed one disorder.

## A. Is Tardive Dystonia Part of TD

Although currently tardive dystonia is regarded to be an integral part of the TD domain, dyskinesia and dystonia might be two distinctly different disorders: the classical description of TD is choreatic movements. Chorea means dance, which refers to fluid motions. Tardive dystonia on the other hand is characterized by a persistent nature of the disorder with a static phenomenology with sustained muscle contractions. Sometimes dystonia impersonates dyskinesia with more fluid-like motions because patients try to actively work against the sustained muscle contraction. Although dystonia has a persistent nature it is often not static. For example, a patient with a torticollis often moves his or her head due to dystonic contractions of different muscle groups or voluntary active antagonist muscles group contraction for correction of the dystonia.

Both tardive variants of dystonia and TD can be caused by dopamine antagonism (Burke *et al.*, 1982; Casey, 2004) which supports a shared domain in spite of the different phenomenology. A possible shared domain of movement disorder is corroborated by a longitudinal cohort study of mostly chronic inpatients. In this population, TD was strongly associated with the severity of tardive dystonia and vice versa (van Harten *et al.*, 2006). There were strong connections between the hyperkinetic syndromes (TD, tardive dystonia, and akathisia) while the hypokinetic Parkinsonism was found to be inversely related to TD and tardive dystonia. Although inversely related the highest prevalence rates of combinations were TD combined with parkinsonism (12.9%) because both side effects are highly prevalent in patients on long-term antipsychotic treatment followed by combinations of TD with tardive dystonia (9.8%) and TD with akathisia 5.2%. (van Harten *et al.*, 1997).

There are two studies evaluating tardive dystonia; in first episode patients and results remain inconclusive whether tardive dystonia occurs in antipsychotic naïve patients with schizophrenia (Pappa and Dazzan, 2009). Current scales do not differentiate between tardive dystonia and TD. Tardive dystonia in antipsychotic naïve patients with schizophrenia could add to the concept that the two phenomenologically different states could indeed be one syndrome.

### 1. *Is akathisia a part of TD*

Akathisia is primarily viewed as an acute dose-dependent syndrome of motor restlessness with a large subjective component (Barnes, 2003). When restlessness persists more than 6 months after the last dose increment the term chronic is proposed (Barnes, 2003). Besides the commonly motor features like marching in place, crossing and uncrossing the legs when sitting, other movement like trunk rocking, respiratory grunting and moaning, complex hand movements as face rubbing, scratching, and rubbing the thighs are thought to be associated with akathisia (Barnes, 2003). These last motor signs are also common in TD possibly leading to misclassification if tardive akathisia is not in the domain of TD. Moreover, similar to dystonia, akathisia has an acute and tardive variety as well. Phenomenologically, the acute and tardive form appears similar; however, with regard to treatment and diagnostic properties the two are distinctly different (Tenback and van Harten, 2011). In fact tardive akathisia and TD are similar with regard to the dosage change of antipsychotic medication. Increasing the dosage often diminish the severity of TD an tardive akathisia while reducing the dosage often increase the severity (often temporarly) of both. Alike tardive dystonia, if akathisia would already manifest itself in antipsychotic naïve patients with schizophrenia it could be because of a shared pathophysiological basis. However, similar to tardive dystonia the presence of akathisia in antipsychotic naïve patients with schizophrenia remains inconclusive (Pappa and Dazzan, 2009). If akathisia had a shared domain with TD, one would expect to manifest itself in antipsychotic naïve patients with schizophrenia (van Harten and Tenback, 2009).

Although currently the tardive form of akathisia could be regarded as a shared domain of TD, most current measures to evaluate movement disorders do not include akathisia thereby affecting incidence and prevalence rates of TD. Moreover, there is also a difference in incidence and prevalence rates of akathisia depending on the type of antipsychotic (Kane *et al.*, 2009).

## B. Scales and Measuring Tardive Dyskinesia and Dystonia

Movement disorder scales are used to measure incidence and prevalence rates. To include or exclude akathisia or dystonia will affect these epidemiological measures. Furthermore, sensitivity and specificity will affect reported incidence and prevalence rates. Also, a lack of standardization towards the rating of the severity of the movement disorders, case definition, and a lack of explicit criteria to differentiate between tardive akathisia, TD, tardive dystonia, stereotypies, spontaneous dyskinesia, and other movements will influence incidence and prevalence rates. Finally, differences in populations at risk or vulnerability due to psychiatric illness or dopaminergic affinity of the dopamine blocker might affect incidence and prevalence rates (van Rossum *et al.*, 2009).

Antipsychotics according to the regulatory authorities should be tested for effectiveness and side effects. In most regulatory studies with antipsychotics in schizophrenia TD was measured with the AIMS (Abnormal Involuntary Movement Scale) (Guy, 1976a) or the ESRS (Extrapyramidal Symptom Rating Scale) is scale which measures TD, parkinsonism, and akathisia in one scale (Chouinard and Margolese, 2005).

None of the above scales used to measure drug-induced movement disorders of antipsychotics in registration dossiers specifically looked at the occurrence of tardive dystonia as a side effect. Moreover, the AIMS does not include the item akathisia. These scales do not differentiate between TD and tardive dystonia; therefore, misclassification might influence rates of TD reported in the literature depending if akathisia and/ or dystonia is reported separately or combined. In meta-analyses reporting on this subject no case definition of TD is mentioned (Correll and Schenk, 2008)

Lastly, sensitivity and specificity influence incidence and prevalence rates. Instrumentally measured movement disorders report higher prevalence rates compared to rates measured with clinical rating scales (Dean *et al.*, 2004).

## C. Mixed Psychiatric Populations

Spontaneous dyskinesia might be a symptom instead of merely a side effect of antipsychotics (van Harten and Tenback, 2009). This might indicate a different etiology or different dispositions to develop TD in different psychiatric diagnostic categories (van Rossum *et al.*, 2009). Also the type of antipsychotic is a determinant in the liability to develop TD (Correll and Schenk, 2008). There are other factors like race and age which seem to affect liability for TD (Tenback *et al.*, 2009). When considering the epidemiology of TD it is important to realize that (Tardive) Dyskinesia itself might be very heterogeneous and incidence and prevalence rates might depend on several risk factors. The course of TD is waxing and waning and the severity of TD can vary over time, during the day and per examiner (Bergen *et al.*, 1984, 1988, 1989).

### *1. Biological basis of spontaneous dyskinesia in schizophrenia*

In schizophrenia, dyskinesia could be a criterion for schizophrenia and thereby more a symptom of the disease than a side effect of dopamine blockade (van Harten and Tenback, 2009). This could suggest a different pathophysiological mechanism than the tardive form induced by dopamine blockade and would justify a separate discussion (van Harten and Tenback, 2009). Furthermore, the adjective Tardive does not apply because it is more an early symptom of schizophrenia and possibly an expression of genetic liability for schizophrenia or psychosis (Koning, 2011; Koning *et al.*, 2011; Mittal *et al.*, 2008).

The biological origin of schizophrenia and movement disorders is likely to reside in a shared dysfunction in the dopamine system. Despite the fact that schizophrenia exhibits a wide clinical variability and heterogeneous genetic architecture, dysfunction in the dopamine system seems to be the final common pathway (Howes and Kapur, 2009).

Schizophrenia is associated with spontaneous dyskinesia in patients never exposed to antipsychotics (Fenton, 2000) and in first-degree family members of patients with schizophrenia (Koning *et al.*, 2010, 2011). In siblings with motor symptoms, dyskinesia was found to be clustered with positive schizotypical symptoms (Koning *et al.*, 2011). In addition, birth cohort studies have shown that infants and children destined to develop schizophrenia have abnormalities in motor development (Cannon *et al.*, 2002; Erlenmeyer-Kimling *et al.*, 2000; Jones *et al.*, 1994). Dyskinesia may therefore form an integral part of the illness and its genetic liability to develop schizophrenia (van Harten and Tenback, 2009) in interplay with environmental factors (van Os and Kapur, 2009). Thus, given the fact that part of the risk for drug-induced movement disorders is thought to be associated with schizophrenia (van Harten and Tenback, 2009; van Os *et al.*, 1997), genes related to schizophrenia are attractive candidates for study in the context of drug-induced movement disorders. Various anatomic and functional mechanism are linked in the striatum and nucleus accumbens related to drug-induced movement disorders suggesting that various combinations of susceptibility genes may converge on synaptic processing in microcircuits leading to EPS or TD. The possible genetic component of shared dopamine dysfunction is illustrated by two meta-analyses; one reporting on spontaneous movement disorder in antipsychotic naïve patients with schizophrenia (Pappa and Dazzan, 2009) and a meta-analysis in which there is a gradual decrease in the prevalence of dyskinesia when comparing antipsychotic-naïve patients with first degree family members, and family members with matched normal controls (Koning *et al.*, 2008). Moreover, a longitudinal study provided evidence for more movement disorders in schizotypical personality disorders than in normal controls, and longitudinally the worsening of movement disorders were correlated with an increase of positive symptoms (Mittal *et al.*, 2008). This strongly suggests that movement disorders are pathogenetically related to psychosis and schizophrenia. It could therefore be imagined that dyskinesia in schizophrenia is a symptom of schizophrenia instead of merely a side effect of antipsychotics (Cortese *et al.*, 2005; van Harten and Tenback, 2009). Just like other signs of schizophrenia, dyskinesia improves after treatment with antipsychotics (Peralta *et al.*, 2010). Thus spontaneous dyskinesia in schizophrenia is not "tardive," and it is not known if spontaneous dyskinesia is pathophysiologically similar to the tardive form, which occurs after antipsychotic treatment.

Dyskinesia as a symptom of schizophrenia could be explained by a theory of dopamine supersensitivity, illustrated by associations of dyskinesia and symptoms in schizophrenia in a large cohort of patients with schizophrenia. In this study, the

main hypothesis is the existence of a pan-dopaminergic D2 hypersensitivity in schizophrenia influenced by exogenous factors as a model to explain the occurrence of TD (Tenback *et al.*, 2010).

Several proxies for the four different dopamine pathways were examined for longitudinal associations with incident TD. The first proxy was early extra pyramidal symptoms (EPS) as a proxy for the nigrostriatale pathway as a risk factor for TD (Tenback *et al.*, 2006a). A second proxy consisted of prolactin-related sexual disturbances as a proxy for the tuberoinfundibular tract independent of the antipsychotic-induced rise in prolactin (Tenback *et al.*, 2006b), and finally, a proxy psychopathological measure of dopaminergic mesolimbic and mesocortical signaling represented by a single Clinical Global Impression overall symptom severity score (CGI) (Guy, 1976b) was used to examine associations between TD onset and change in CGI scores, hypothesized to reflect underlying changes in neurochemical signaling in the mesolimbic and mesocortical pathways (Tenback *et al.*, 2007). This resulted in positive longitudinal associations of all proxies for the different dopamine tracts and the emergence of TD.

Thereafter, calculating the incidence and persistence of TD and EPS in the same cohort of patients with schizophrenia, treated predominantly with SGA, over a period of 2 years, assessed the morbidity force of movement disorder. In line with previous publications, incidence rates of TD and EPS were considerably lower in the SGA era. However, once emerged, these disorders prove highly persistent, suggesting strong moderator effects of underlying pre-disposing factors. To explain these strong moderator effects, a hypothesis of a pandopaminergic supersensitivity was proposed where TD and EPS are core symptoms of schizophrenia and possible markers for the course of the disease, and where gradually increasing dopamine (D(2)) receptor sensitivity can be considered as a marker for schizophrenia disease severity (Tenback *et al.*, 2010).

These findings are corroborated by a study using positron emission tomography that patients showing the highest degree of D2 receptor upregulation after using Q24 antipsychotics develop TD (Silvestri *et al.*, 2000). The association with schizophrenia is illustrated by a twin study showing that the unaffected co-twins of patients had increased caudate D2 density compared to healthy twin-pair controls, a finding that implies that D2 receptor up-regulation is related to the liability for schizophrenia (Hirvonen *et al.*, 2005) and, finally, the finding that first-degree family members of patients with schizophrenia displayed more dyskinesia and parkinsonian signs than a healthy matched control group (Koning *et al.*, 2008).

Contrary to the debate on the specificity of overall neurological signs for schizophrenia (Boks *et al.*, 2000), the diagnostic specificity of TD in antipsychotic-naïve patients in schizophrenia is more apparent (Chatterjee *et al.*, 1995; Fenton, 2000; McCreadie *et al.*, 2003). Indicators of nigrostriatal dysfunction such as TD and other EPS may therefore be considered as an integral part of the

underlying disease (van Harten and Tenback, 2009) and its genetic aetiology and could be considered as a schizophrenia-associated endophenotype (Gottesman and Gould, 2003).

However, when comparing studies in a populations of patients with schizophrenia reporting on both dyskinesia (never treated with antipsychotics) and TD (treated with antipsychotics), both present more or less similar prevalence rates in patients for the never exposed and in those exposed to antipsychotics groups (McCreadie *et al.*, 1996; Owens *et al.*, 1982), although not all studies endorse these findings (McCreadie and Ohaeri, 1994). The data seem to suggest that antipsychotics in schizophrenia merely have a potentiating effect.

Although further research should validate the concept of gradually increasing dopamine (D(2)) receptor sensitivity in schizophrenia, early EPS and Dyskinesia could be useful early markers for disease prognosis, and thus make it possible to consider implications with regard to pharmacological treatment options, early treatment intervention, and expected burden of care (Ascher-Svanum *et al.*, 2008; Caroff *et al.*, 2011; Murray and Van Os, 1998).

## IV. Conclusion

TD in diagnostically mixed populations treated with dopamine blockers may differ from spontaneous dyskinesia in antipsychotic naïve patients with schizophrenia. The incidence of TD in the era of SGA with a lower affinity to the dopamine D2 receptor seems generally lower but remains a clinical concern especially since it might be related to a worse clinical outcome.

Prevalence rates of TD remain high, even in antipsychotic naïve patients, and there is an indication that switching dopamine blockers with high affinity to the Dopamine D2 receptor to blockers with a lower affinity to the D2 receptor might improve symptoms of TD. It is not clear if this also improve clinical outcome.

There is a large quantity of literature on risk factors for TD. There are however few "hard" risk factors. Age, early EPS, and ethnicity are probable risk factors for TD. There are some promising genes that show an association with TD. These genes are either related to an increased dose linked to the impaired metabolism or linked to hypotheses with regard to the pathophysiological basis of TD. Some caution should be exerted because these are cross-sectional associations reported in the literature and do not differentiate in the mixture between onset and persistence of TD.

TD remains an interesting field for clinicians and research especially in psychiatry where it is one of the few symptoms that can be measured objectively.

## Acknowledgment

D.E. Tenback and P.N. van Harten would like to acknowledge P. Roberto Bakker for his revision of the genetic epidemiology.

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# GENETICS OF TARDIVE DYSKINESIA

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Tardive dyskinesia (TD) is one of the most serious adverse side effects of antipsychotic drugs and is an important topic of pharmacogenetic studies. Since there is a genetic susceptibility for developing this adverse reaction, and given that it is hard to predict its development prior to or during the early period of medication, the genetic study of TD is a promising research topic that has a direct clinical application. Moreover, such studies would improve our understanding of the genetic mechanism(s) underlying abnormal dyskinetic movement. A substantial number of case-control association studies of TD have been performed, with numbers of studies focusing on the genes involved in antipsychotic drug metabolism, such as those for cytochrome P450 (*CYP*) and oxidative stress related genes as well as various neurotransmitter related genes. These studies have produced relatively consistent though controversial findings for certain

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00010-9




0074-7742/10 $35.00

polymorphisms such as *CYP2D6**10, *DRD2* Taq1A, *DRD3* Ser9Gly, *HTR2A* T102C, and *MnSOD* Ala9Val. Moreover, the application of the genome-wide association study (GWAS) to the susceptibility of TD has revealed certain associated genes that previously were never considered to be associated with TD, such as the rs7669317 on 4q24, *GLI2* gene, GABA pathway genes, and *HSPG2* gene. Although a substantial number of genetic studies have investigated TD, many of the positive findings have not been replicated or are inconsistent, which could be due to differences in study design, sample size, and/or subject ethnicity. We expect that more refined research will be performed in the future to resolve these issues, which will then enable the genetic prediction of TD and clinical application thereof.

## I. Introduction

Tardive dyskinesia (TD) is a serious adverse side effect that is occasionally experienced by schizophrenic patients who are treated with antipsychotic drugs. Although the prevalence rates are difficult to estimate and have reportedly differed between studies, a meta-analysis including 39,187 subjects from 76 studies found an overall prevalence of 24.2% (Yassa and Jeste, 1992). The most typical sign of TD is involuntary orofacial dyskinesia, but the trunk and extremities may also be affected. TD is generally caused by antipsychotics, and particularly first-generation antipsychotics (FGAs), but sometimes also second-generation antipsychotics (SGAs). Although many SGAs have been developed and are increasingly used, FGAs are still extensively prescribed due to factors such as the lack of any significant differences in the efficacy of the two generations of antipsychotic (Lieberman, 2007), the side effects of SGAs (such as metabolic syndrome), and the lower acquisition costs of FGAs.

The causes of the TD are deemed multifactorial; many multiple demographic causes including the age, gender, dosage, ethnicity, and duration of exposure to antipsychotics have been proposed, and several pathophysiological causes have also been proposed, none of which has been considered conclusive. Several biological mechanisms underlying the pathophysiology of TD have been proposed, including dopamine receptor hypersensitivity (Tarsy and Baldessarini, 1977), serotonergic dysfunction (Meltzer, 1994), γ-aminobutyric acid (GABA) insufficiency (Casey *et al.*, 1980), and disturbances of antioxidative protection (Andreassen and Jorgensen, 2000). However, the pathophysiology of TD remains poorly understood.

Many studies have provided evidence that TD involves genetic and familial causes. Specifically, it has been found that TD occurs only in some patients taking antipsychotics, and that such occurrences involve a familial tendency, thus

indicating a biological or genetic factor (Tamminga *et al.*, 1990; Yassa and Ananth, 1981). This background has prompted many genetic studies of TD, which mainly involve pharmacogenetic investigations of antipsychotics. Another reason why many studies have investigated TD pharmacogenetics is that TD is the type of side effect that is potentially irreversible and it is very hard to predict who it will affect. Furthermore, TD causes patients serious distress and leads to noncompliance with pharmacotherapy. Elucidating the details of the genetic susceptibility to this side effect would make prescription after genotyping and biomarker-guided prediction possible (Ozdemir *et al.*, 2006). In the future, it may become possible to calculate the probability of developing TD by considering the presence of certain associated variables (i.e., genes and demographic parameters). Moreover, the pharmacogenetic study of TD will contribute to discovery of the genetic mechanism underlying abnormal dyskinetic movement and movement disorders.

The candidate genes that are thought to determine susceptibility to TD are cytochrome P450 (CYP), diverse neurotransmitter, and oxidative-stress-related genes. The medication response is very closely related to the drug metabolism, and CYP genes have been investigated extensively. In addition, the neurotransmitter-related genes, and especially those related to dopamine and serotonin, have been studied substantially because these neurotransmitters are deemed to be the targets of antipsychotics. Several recent studies of oxidative-stress-related genes have provided evidence of a relationship between TD and oxidative stress. Moreover, numerous pharmacogenetic studies have investigated genes related to neurotrophic factors, opioid receptors, estrogen receptors, the GABA pathway, and the glutaminergic pathway. Fig. 1 shows hypothetical genetic factors contributing to TD.

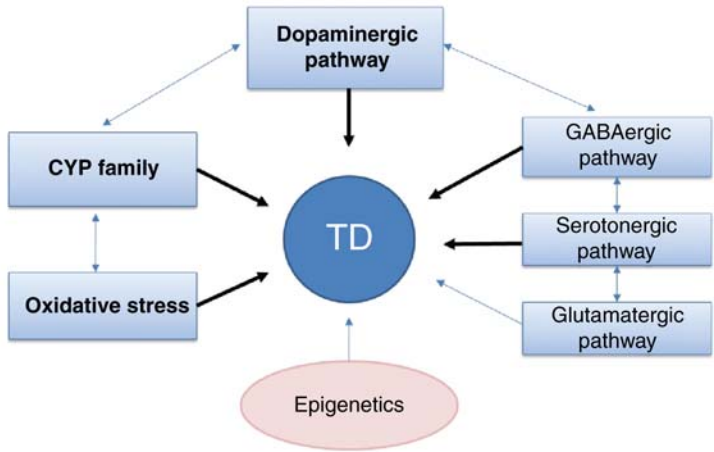


FIG. 1. Genetic factors contributing to TD. Bold arrows represent having replicated evidences by multiple studies. TD, tardive dyskinesia; CYP, cytochrome P450. GABA, gamma-aminobutyric acid.

## II. Genes Involved in Pharmacokinetics

The metabolism of antipsychotic drugs is a crucial determinant of their therapeutic and adverse effects. The contribution of pharmacokinetic factors is important to the clinical outcome of antipsychotic treatment. Antipsychotic drugs are metabolized and distributed by various enzymes. For example, since perphenazine is metabolized extensively by CYP2D6, variants of CYP2D6 may substantially influence the exposure of perphenazine to patients. It is hypothesized that reduced metabolism of antipsychotics by a CYP variant resulting in deficient enzymatic activity can increase the possibility of TD development due to increased exposure to the drug.

### A. Phase I enzymes (CYP family)

Phase I oxidation is a fairly significant metabolic process for antipsychotics. Most antipsychotics undergo extensive metabolism by various enzymes of the CYP family, which play an important role in the elimination of the drugs, thus influencing their effectiveness and adverse effects. Poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers, and ultrarapid metabolizers (UMs) are phenotypes of persons having defective, reduced, normal, and duplicate copies of the *CYP* genes, respectively.

CYP2D6 is the major metabolic factor of many typical antipsychotics, and is the most extensively investigated enzyme with regard to genetic polymorphisms. Coding of the *CYP2D6* gene is highly variable. Among 90 mutations, four polymorphisms (*3, *4, *5, and *6) cover most of the inactive alleles (98%) in Caucasians (Bradford, 2002). Some gene duplications (or multiplications) are responsible for the UM phenotype. The allele frequencies of *CYP2D6* polymorphisms differ with ethnicity, such as PMs representing 7–10% of Caucasians but only 1–2% of Asians (Kubota *et al.*, 2000). This suggests that the efficacy and the severity of adverse reaction to medication depend on ethnicity. Genetically determined CYP enzyme dysfunction and the ensuing accumulation of drug metabolites contribute significantly to the development of TD. The adverse effects of antipsychotics are known to be associated with the CYP2D6 enzyme (Arthur *et al.*, 1995; de Leon *et al.*, 2005a). Thus, if the metabolism of haloperidol is extremely reduced by PMs, then the therapeutic dosage should be reduced accordingly in these patients (Kirchheiner *et al.*, 2004).

It was recently observed that the CYP2D6 phenotype does not predict the effectiveness of risperidone, but rather predicts the metabolic rate and side effects of the drug (de Leon *et al.*, 2005a; Kakihara *et al.*, 2005; Riedel *et al.*, 2005). Several associations between the *CYP2D6* gene and TD have been reported, showing that

genetic variants for reduced CYP2D6 metabolism are particularly associated with TD development. For Asian populations, the frequency of *CYP2D6*-genotypic PMs (*3, *4, *5, and *6) is rare. However, IMs who are *CYP2D6**10 allele carriers with reduced enzymatic activity are very common, representing 40–50% of the Asian population (Lee *et al.*, 2006).

One Japanese study found that the *CYP2D6**10 genotype plays a role in the development of moderate or severe abnormal movements (Ohmori *et al.*, 1998). A Chinese study showed an increased frequency of the *CYP2D6**10 allele in female patients with TD (Lam *et al.*, 2001), and another Chinese study found an association between *CYP2D6**10 C188T polymorphism and TD (Liou *et al.*, 2004a). Fu *et al.* (2006) observed significant excess of the T allele of the *CYP2D6* C100T polymorphism in Chinese schizophrenic TD patients.

Meanwhile, Kobylecki *et al.* (2009) showed that TD develops more frequently in Danish PMs patients (subjects who carry two of the following mutation alleles: *3, *4, *5, and *6). Previous research on Caucasians concluded that heterozygous carriers of PM alleles (*1/*3 or *1/*5) of *CYP2D6* exhibited an increased susceptibility to the development of TD (Kapitany *et al.*, 1998). Antipsychotic drug exposure, genotype, and cigarette smoking interaction were significant factors for schizophrenia patients with the *CYP2D6**1/*3, *4 genotype (Ellingrod *et al.*, 2002). Of smokers carrying the *CYP2D6**1/*3, *4 genotype, 78% had TD, compared to 20–33% of patients in other groups, which suggests that *CYP* PM allele carriers shunt antipsychotic metabolism through other pathways that are induced by cigarette smoking.

Screening tests for 19 alleles using the Affymetrix *CYP* GeneChip system revealed that the probability of developing TD was moderately higher in Korean male schizophrenic patients with at least one decreased or loss-of-function allele than in those with only wild-type alleles (Nikoloff *et al.*, 2002). A recent meta-analysis investigating the association between *CYP2D6* polymorphism and TD revealed that subjects with loss-of-function alleles (PMs) are vulnerable to the development of TD (Patsopoulos *et al.*, 2005).

CYP1A2 is a major metabolic iso-enzyme of clozapine and olanzapine (Eiermann *et al.*, 1997; Ring *et al.*, 1996). Three variants of *CYP1A2* polymorphisms (*1C, *1K, and *11) show decreased activity (Murayama *et al.*, 2004; Sachse *et al.*, 1999). However, the *CYP1A2* polymorphism does not markedly influence the metabolism of clozapine (Kootstra-Ros *et al.*, 2005), although delayed therapeutic responses have been reported in patients with the UM phenotype (Eap *et al.*, 2004; Ozdemir *et al.*, 2001). An association between *CYP1A2* variants and the genetic risk factors for the development of TD has also been reported by several research groups. For example, the C-allele frequency of the *CYP1A2* C163A single-nucleotide polymorphism (SNP) was significantly higher in patients with TD than in those without TD (Fu *et al.*, 2006). In addition, the mean score on the abnormal involuntary movement scale (AIMS) was 2.7- and 3.4-fold higher in carriers of the C/C genotype (which is associated with reduced CYP1A2 inducibility) than in those with

the A/C or A/A genotype, respectively (Basile *et al.*, 2000). Furthermore, Tiwari *et al.* (2005a) reported an association between *CYP1A2**1C polymorphism and TD.

Variants of the *CYP3A4* enzyme are also related to the metabolism of most antipsychotics. However, only *CYP3A4**17 and *18A exhibit functional variety, with a decreased or increased activity, respectively (Dai *et al.*, 2001). No relationship between the *CYP3A5*, *CYP2C9*, and *CYP2C19* variants and the level of efficacy or adverse reaction to antipsychotics has been reported (Fang and Gorrod, 1999; King *et al.*, 2004; Sim *et al.*, 2006). Research on the *CYP3A4* and *CYP3A5* genes has failed to yield any association with TD (de Leon *et al.*, 2005b; Tiwari *et al.*, 2005b).

The first pharmacogenetic microarray-based test (AmpliChip) was recently approved for clinical use (de Leon, 2006). The AmpliChip supplies a comprehensive coverage of gene variations for the *CYP2D6* and *CYP2C19* genes, which play a pivotal role in the metabolism of approximately 25% of all prescribed drugs. However, the clinical effectiveness and cost-effectiveness of testing for CYP in schizophrenic patients treated with antipsychotics has not been confirmed (Fleeman *et al.*, 2010). Future genetic studies of *CYP* genes should use larger samples, and detailed data regarding patient selection, genotype information, environmental factors (smoking, concomitant medications, medication adherence, and ethnicity), and pharmacokinetic parameters should be provided to clarify the cost-effectiveness of testing for CYP. The adherence, relapse, quality of life, and life expectancy of patients with schizophrenia also need to be investigated (Fleeman *et al.*, 2010). Table I shows studies reported significant association between *CYP* genes and TD.

### B. Phase II Enzymes

Phase II enzymes are responsible for the inactivation of drug metabolites via conjugation reactions. *N*-acetyltransferases, thiopurine *S*-methyltransferases, uridine diphosphate glucuronosyltransferases, and glutathione *S*-transferases (GSTs) are major enzymes involved in Phase II reactions. Some researchers have suggested that Phase II enzyme variants contribute to treatment variability and disease pathogenesis, especially those related to toxic environmental compounds (Cascorbi, 2006). Genetic differences underlying differences in drug metabolism have important implications for determining the appropriate therapeutic dosage and may also be related to variations in the drug toxicity. Pharmacogenetic studies of pharmacokinetic factors have yielded the most informative and clinically useful results in clinical psychiatry. Genetic information on the metabolic status of the individual patient may be beneficial to determining the optimal antipsychotic treatment in clinical practice. It has been estimated that pretreatment metabolic determination may decrease adverse reactions by 10–20% and improve drug efficacy by 10–15% (Ingelman-Sundberg, 2004). Genetic variants of some other Phase II enzymes are discussed in the section on oxidative-stress-related genes.

Table I
STUDIES REPORTED SIGNIFICANT ASSOCIATION BETWEEN *CYP* GENES AND TD.

| Genes investigated | SNP (genotype or allele) | Findings | Ethnicity | Investigators, year |
|---|---|---|---|---|
| ***CYP2D6*** | *1, *3, *4, and *5 alleles | *1/*3 and *1/*5 genotypes: association with TD | Austrian | Kapitany *et al.*, 1998 |
| | *1, *3, and *4 alleles | *1/*3, *1/*4: association with TD | Caucasian | Ellingrod *et al.*, 2002 |
| | *3, *4, and *10 alleles | *10 allele: association with TD and its severity | Japanese | Ohmori *et al.*, 1998 |
| | *10 allele | *10 allele and genotype: association with TD only in females | Chinese | Lam *et al.*, 2001 |
| | *10 C188T | *10 C188T: association with TD only in males | Chinese | Liou *et al.*, 2004a |
| | C100T of CYP2D6 and C163A of CYP1A2 gene | T allele of 2D6 C100T: association with TD<br>C allele of 1A2 C163A: association with TD | Chinese | Fu *et al.*, 2006 |
| | 19 alleles of CYP2D6 gene | Decreased or loss of function allele: association with TD (screened by Affymetrix GeneChip) | Korean | Nikoloff *et al.*, 2002 |
| | *2, *6, *7, *10, and other alleles | Loss of function alleles: association with TD (meta-analysis) | Caucasian and Asian | Patsopoulos *et al.*, 2005 |
| ***CYP1A2*** | 734 C/A | CC genotype: association with severity of TD | Caucasian and African-American | Basile *et al.*, 2000 |
| | *1C and *1F alleles | *1C: association with severity of TD | Indian | Tiwari *et al.*, 2005a |

CYP, cytochrome P450; TD, tardive dyskinesia; SNP, single-nucleotide polymorphism.

## III. Genes Involved in Pharmacodynamics

Pharmacogenetic studies of pharmacodynamic factors have been conducted in order to validate therapeutic targets. Neurotransmitter systems in the brain are considered to be altered in patients with schizophrenia, and hence have been targets of antipsychotic therapy. Antipsychotics have diverse affinities for each neurotransmitter receptor, including dopamine, serotonin, adrenergic, glutamate, histamine, and muscarine receptors. Therefore, the pharmacodynamic properties of antipsychotics are responsible for both their side effects and their therapeutic effects. The main candidate genes of antipsychotic-drug-induced TD are dopamine-, serotonin-, GABA-, and glutamatergic pathway genes. Table II summarized the studies reporting significant association between dopamine or serotonin pathway genes and TD.

### A. Dopamine-related Genes

The dopaminergic system is considered to be an important neurotransmitter system for mediating the activity of antipsychotics. Dopamine dysfunctions of the brain have been the major pathological findings in schizophrenia research, and most antipsychotic drugs appear to have a preferential affinity for dopamine receptors. Therefore, dopaminergic receptor genes have become the most widely investigated in studies of TD pharmacogenetics.

#### 1. *Dopamine D2 Receptor*

Numerous genetic case-control association studies of dopamine receptor genes have been performed. Chen *et al.* (1997) performed an association study of Taiwanese schizophrenic patients and found that the frequency of the TaqI A2/A2 genotype of the dopamine D2 receptor (*DRD2*) gene was higher in female patients with TD than in those without TD. Two recent meta-analysis studies yielded similar results. Zai *et al.* (2007a) revealed higher A2 allele and A2/A2 genotype frequencies in TD patients, and Bakker *et al.* (2008) reported the risk-increasing effects of the A2 variant (using the A1 variant as a reference category) and of A2/A2 homozygotes (using A1/A1 as a reference category). Other SNPs of the DRD2 gene have also been investigated. Liou *et al.* (2006a) found that B2 homozygotes of Taq1B and A2 homozygotes of Taq1A are associated with susceptibility to TD. Hori *et al.* (2001) found a marginally significant association between the 141C Ins/Del polymorphism and the total AIMS score. Zai *et al.* (2007b) found that genotype frequencies of the functional polymorphisms (i.e., C957T and the adjacent C939T) are significantly associated with TD. Our group also performed an association study using five *DRD2* polymorphisms for TD in

Table II
Studies Reported Significant Association Between Dopamine- and Serotonin-Related Genes and TD.

| Genes investigated | SNP (genotype or allele) | Findings | Ethnicity | Investigators, year |
|---|---|---|---|---|
| ***DRD2*** | TaqI A | A2/A2 genotype: association with TD in female | Chinese | Chen *et al.*, 1997 |
| | TaqI A and -141 Ins/Del | A2 allele and A2/A2 genotype: association with TD | Meta-analysis | Zai *et al.*, 2007a |
| | TaqI A of DRD2 and other genes (COMT, CYP1A2, and MnSOD) | A2 allele and A2/A2 genotype: association with TD | Meta-analysis | Bakker *et al.*, 2008 |
| | -141C Ins/Del, TaqI B, TaqI D, S311C, and TaqI A | B2/B2 genotype of TaqIB and A2/A2 genotype of TaqIA: protective haplotype against TD | Chinese | Liou *et al.*, 2006a |
| | Ser311Cys TaqI and A-141C Ins/Del | -141 Ins/Del: more severe TD | Japanese | Hori *et al.*, 2001 |
| | 12 SNPs | C957T and C939T: association with TD | Caucasian and African-American | Zai *et al.*, 2007b |
| ***DRD3*** | Ser9Gly | Gly/Gly: association with TD | Scottish | Steen *et al.*, 1997 |
| | Ser9Gly | Gly allele: association with TD | Jewish | Segman *et al.*, 1999 |
| | Ser9Gly | Gly/Gly genotype: more severe TD | Caucasian and Jewish | Lerer *et al.*, 2002 |
| | Ser9Gly | Gly allele: more severe TD | Caucasian and other ethnicities | de Leon *et al.*, 2005 |
| | Ser9Gly | Ser/Gly genotype: more severe TD | Chinese | Liao *et al.*, 2001 |

(*continued*)

Table II (*continued*)

| Genes investigated | SNP (genotype or allele) | Findings | Ethnicity | Investigators, year |
|---|---|---|---|---|
| | Ser9Gly | Gly/Gly genotype: association with TD | Korean | Woo *et al.*, 2002 |
| | Ser9Gly | Gly allele: association with TD | Meta-analysis | Bakker *et al.*, 2006 |
| | Ser9Gly | Gly allele: more severe limb-truncal TD | Russian | Al Hadithy *et al.*, 2009 |
| | 34 SNPs of *DRD3* and 14 SNPs of *BDNF* | rs905568 G allele: protective against TD | Caucasian | Zai *et al.*, 2009b |
| | Ser9Gly and *HTR2C* | Gly allele: more severe TD | Greek | Rizos *et al.*, 2009 |
| ***DRD4*** | SNPs from *DRD2, DRD3, DRD4*, and *HTR2A* | Short allele of VNTR polymorphism in exon 3: association with TD | Italian | Lattuada *et al.*, 2004 |
| | SNPs from *DRD1, DRD2, DRD3, DRD4, DAT*, and *COMT* | DRD4 120 bp duplication marker: association with TD | Indian | Srivastava *et al.*, 2006 |
| | 5 SNPs | Haplotypes consisting of 4 SNPs (rs3758653, rs916457, rs762502, rs11246226): association with TD in males | Caucasian | Zai *et al.*, 2009a |
| ***COMT*** | SNPs from *DRD1, DRD2, DRD3, DRD4, DAT*, and *COMT* | 408C/G and Val158Met genotype: association with TD | Indian | Srivastava *et al.*, 2006 |
| | Val158Met of *COMT* and other genes (*DRD2, CYP1A2*, and *MnSOD*) | Val/Met genotypes: protection against TD | Meta-analysis | Bakker *et al.*, 2008 |

| | | | | |
|---|---|---|---|---|
| ***HTR2A*** | T102C, A-1438G, His452Tyr | 102C and -1438G alleles: association with TD<br>102CC and -1438GG genotypes: association with TD | Jewish | Segman *et al.*, 2001 |
| | T102C | CC genotype: association with TD | Italian | Lattuada *et al.*, 2004 |
| | T102C | T allele and T/T genotype: associated with non-occurrence of TD | Chinese Singaporean | Tan *et al.*, 2001 |
| | T102C and His452Tyr | T102C genotype: association with TD | Mixed ethnicity (Caucasian, Jewish, and Asian) | Lerer *et al.*, 2005 |
| | -1438G/A | AA genotype: related to TD | Turkish | Boke *et al.*, 2007 |
| ***HTR2C*** | Cys23Ser of *HTR2C* and *DRD3* | Ser allele: associated with TD | Jewish | Segman *et al.*, 2000 |
| | -759C/T, -697C/G | -697C allele: association with TD | Chinese | Zhang *et al.*, 2002 |

TD, tardive dyskinesia; SNP, single-nucleotide polymorphism; DRD2, dopamine D2 receptor; COMT, catechol-*O*-methyltransferase; CYP, cytochrome P450; MnSOD, manganese superoxide dismutase; DRD3, dopamine D3 receptor; DRD4, dopamine D4 receptor; VNTR, variable number of tandem repeat; DAT, dopamine transporter; bp, base pair; BDNF, brain-derived neurotrophic factor; HTR, 5-hydroxytryptamine receptor.

Korean schizophrenic patients. However, we did not find any association between SNPs or haplotypes and TD (Park *et al.*, 2011).

2. *Dopamine D3 Receptor*

The dopamine D3 receptor (*DRD3*) is located in the brain areas that control motor function, and DRD3 antagonism has been shown to exacerbate locomotor activity in the brain (Accili *et al.*, 1996). In addition, antipsychotics with comparatively low DRD3 affinity, such as clozapine, are reported to cause significantly rarer movement disorders (Kapur and Seeman, 2001). The *DRD3* gene has been suggested as a susceptibility factor for TD, and the Ser9Gly SNP of this gene has been investigated extensively, resulting in many positive findings (Al Hadithy *et al.*, 2009; de Leon *et al.*, 2005b; Lerer *et al.*, 2002; Liao *et al.*, 2001; Rizos *et al.*, 2009; Segman *et al.*, 1999; Steen *et al.*, 1997; Woo *et al.*, 2002). A meta-analysis of this polymorphism produced results in line with those of other studies (Bakker *et al.*, 2006). However, negative results have also been reported (Lee *et al.*, 2008; Rietschel *et al.*, 2000). Zai *et al.* (2009b) found that TD was associated with rs905568 SNP but not with Ser9Gly after investigating 34 SNPs of the *DRD3* gene and 14 SNPs of the brain-derived neurotrophic factor (BDNF) gene. Furthermore, a very recent meta-analysis found no association between TD and Ser9Gly using stratified analysis and meta-regression (Tsai *et al.*, 2010a). Consequently, the once widely accepted association between Ser9Gly and TD has been recently become equivocal.

3. *Dopamine D4 Receptor*

Fewer studies have investigated how the development of TD is associated with the dopamine D4 receptor (*DRD4*) gene than with *DRD2* and *DRD3*. Lattuada *et al.* (2004) reported a marginally significant association between the short allele of the *DRD4* variable number of tandem repeat (VNTR) polymorphisms in exon III and TD, but Segman *et al.* (2003) reported that there was no such association. Srivastava *et al.* (2006) found that a 120-bp duplication marker that is 1.2-kb upstream of the initiation codon of the DRD4 gene is associated with TD. However, our group could find no association between another SNP of *DRD4* (–521C/T) and TD (Lee *et al.*, 2007a). Zai *et al.* (2009a) examined the five SNPs of the *DRD4* gene and showed that the haplotype of four tag SNPs (only the VNTR in exon III was excluded in the haplotype) was associated with TD. Therefore, replication studies are necessary to determine the association (if any) between TD and the other SNPs of *DRD4*, as well as the VNTR in exon III.

4. *Other Dopamine-Related Genes*

Other dopamine-related genes have been studied for possible associations with TD. Srivastava *et al.* (2006) reported no association when they investigated DRD1

polymorphisms and a polymorphism in the dopamine transporter in relation to TD. Similarly, the monoamine oxidase A and B genes involved in dopamine degradation were not found to contribute to TD (Matsumoto *et al.*, 2004). Furthermore, there is no disagreement as to the association between TD and the dopamine degradation enzyme catechol-*O*-methyltransferase (COMT) genes (Bakker *et al.*, 2008; Kang *et al.*, 2008a; Lai *et al.*, 2005; Matsumoto *et al.*, 2004; Srivastava *et al.*, 2006). Tyrosine hydroxylase (TH) is the rate-limiting enzyme in dopamine biosynthesis. Because the *TH* Val81Met polymorphism is located in the amino-terminal regulatory domain of the tetrameric enzyme, it is a candidate marker for susceptibility to dopamine-related traits such as TD. However, Lee *et al.* (2009) did not find any significant association between the *TH* Val81Met polymorphism and TD.

## B. Serotonin-Related Genes

The serotoninergic system is also considered to be related to schizophrenia due to the hallucinogenic properties of a serotonin antagonist, lysergic acid diethylamide. Recent attention paid to the function of the serotoninergic system with regard to the action of antipsychotics has been based mainly on many atypical antipsychotics (e.g., clozapine, olanzapine, quetiapine, risperidone, and ziprasidone) being potent 5-hydroxytryptamine 2A receptor antagonists and weaker DRD2 antagonists (Meltzer, 1999).

In this context, many pharmacogenetic studies of the side effects of antipsychotic drugs have investigated serotonin-related genes.

### 1. *Serotonin 2A Receptor Gene*

Serotonin inhibits dopamine function, which supports the hypothesis that the serotonergic system is involved in the pathogenesis of TD. Tan *et al.* (2001) and Segman *et al.* (2001) were the first to report the existence of an association between serotonin 2A receptor gene (*HTR2A*) T102C polymorphisms and TD. However, although their findings were not replicated in subsequent studies (Basile *et al.*, 2001; Herken *et al.*, 2003), they were eventually confirmed by Lerer *et al.* (2005) in a combined meta-analyses controlling for age, which is an important factor in the development of TD. A logistic regression analysis conducted by Boke *et al.* (2007) revealed that another SNP (–1438A/G) of the *HTR2A* gene is associated with TD. Hsieh *et al.* (2010) found that the *HTR2A* T102C polymorphism was associated with susceptibility to TD, and especially the limb-truncal subtype in Taiwanese schizophrenia patients. However, the –1438A/G SNP was not associated with TD in either Russian (Al Hadithy *et al.*, 2009) or African-Caribbean (Wilffert *et al.*, 2009) schizophrenia patients. Interestingly, Wilffert *et al.* (2009) reported that the combination of 23Ser of the serotonin 2C receptor (*HTR2C*) and –1438A of *HTR2A* carriership increased the risk of TD only in male African-Caribbean schizophrenic patients.

2. *Serotonin 2C Receptor*

Cys23Ser and –697C/G polymorphisms in serotonin 2C receptor (*HTR2C*) gene have been reported to be associated with TD by some authors (Segman *et al.*, 2000; Zhang *et al.*, 2002) but not by others (Deshpande *et al.*, 2005; Rietschel *et al.*, 1997). Al Hadithy *et al.* (2009) reported an association between Cys23Ser of *HTR2C* and limb-truncal TD, but not orofaciolingual TD among 146 Russian schizophrenia patients.

3. *Serotonin-Transporter-Linked Promoter Region*

Studies of serotonin transporter gene polymorphisms have failed to find any association with TD (Chong *et al.*, 2000; Herken *et al.*, 2003; Hsieh *et al.*, 2010; Ohmori *et al.*, 2002).

## C. GABA-Related Genes

Reduced activity in a striatal GABA neurons has been suggested as the one of the possible causes of TD (Kulkarni and Naidu, 2003). In an animal study, Gunne *et al.* (1984) found that decreased GABA activity in the substantia nigra was correlated with enhanced oral movement in rats and with neuroleptic-induced dyskinesia in monkeys. Delfs *et al.* (1995) reported elevated levels of mRNA encoding glutamic acid decarboxylase (the rate-limiting enzyme in GABA synthesis) in the striatum and pallidum of adult rats after long-term haloperidol treatment, suggesting that decreased GABA transmission plays a critical role in the motor side effects associated with long-term antipsychotic therapy.

Inada *et al.* (2008) conducted a genome-wide association study (GWAS) to identify the pathway(s) in which genetic variations influence susceptibility to neuroleptic-induced TD. They suggested that the GABA receptor signaling pathway is involved in the genetic susceptibility to treatment-resistant TD. In their study, eight genes (*ABAT*, *ALDH9A1*, *GABRA3*, *GABRA4*, *GABRB2*, *GABRAG3*, *GPHN*, and *SLC6A11*) contained polymorphisms with gene-based corrected allelic probability values of $<0.05$. Associations were replicated in an independent sample of 36 patients with TD and 136 patients without TD for polymorphisms in *SLC6A11* (GABA transporter 3), *GABRB2* ($\beta$-2 subunit of the GABA-A receptor), and *GABRG3* (c-3 subunit of the GABA-A receptor) (Inada *et al.*, 2008). Our group very recently attempted to confirm this association between these three GABA-related genes (*SLC6A11*, *GABRB2*, and *GABRG3*) and TD, but were only able to replicate their finding for rs4684742 of *SLC6A11* (Lee *et al.*, 2011). However, another GWAS using 2580 SNPs in 118 candidate genes selected from the literature including GABA pathways, with the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study, did not find any significant association between GABA-related genes and TD (Tsai *et al.*, 2010b).

### D. Glutamatergic Genes

It has been hypothesized that increased release of glutamate due to prolonged antipsychotics treatment may result in an excitotoxic lesion in basal ganglia leading to TD (McGeer and McGeer, 1976). Animal studies reported that haloperidol-induced spontaneous oral dyskinesias (i.e., vacuous chewing movement, VCM) was attenuated when a co-treatment with the anti-excitotoxic GM1 ganglioside was provided (Andreassen and Jorgensen, 1994) and memantine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, inhibited the development of VCM in rats (Andreassen *et al.*, 1996). These findings strongly indicate that NMDA receptor-mediated excitotoxicity is involved in antipsychotic-induced VCM development. Meshul *et al.* (1994) reported that treatment with haloperidol for 1 month results in an increase in the mean percentage of striatal asymmetric synapses containing a perforated postsynaptic density and that these synapses are glutamatergic. The glutamate release may be modulated by dopamine receptors located on corticostriatal terminals and the release of glutamate can be increased due to haloperidol-induced blockage of DRD2 receptors (Yamamoto and Davy, 1992). Therefore, glutamatergic candidate genes have been implicated in the etiology of TD. Three polymorphisms (−200T/G, 366C/G and 2664C/T) of the glutamate receptor ionotropic NMDA 2B (*GRIN2B*) gene were analyzed in Chinese schizophrenic patients; however, no association with susceptibility to, or severity of TD was found (Liou *et al.*, 2007).

## IV. Oxidative-Stress-Related Genes

Neuronal degeneration by oxidative stress has been suggested as a mechanism for TD pathogenesis. Excessive reactive oxygen species (ROS) produced by an imbalance between free-radical metabolism and the antioxidant defense mechanism can interact with lipids, proteins, and nucleic acids, resulting in cellular dysfunction or cell death (Halliwell, 1997). Neuronal cells appear to be highly susceptible to oxidative damage, and several studies have found that elevated lipid peroxidation in the cerebrospinal fluid (CSF) might be related to the development and severity of TD (Brown *et al.*, 1998; Lohr *et al.*, 1990a). Detoxification enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase that function as cellular defense mechanisms against oxidative stress have been regarded to be related to TD susceptibility (Rao and Balachandran, 2002). Animal studies have also suggested that oxidative stress plays a role in the pathogenesis of TD (Sagara, 1998). Long-term exposure to antipsychotics increases dopamine turnover, which results in the excessive production of oxidative

metabolites, especially in dopamine-rich brain regions such as the basal ganglia. The oxidative metabolites, which are dopamine quinones and hydrogen peroxide, lead to the formation of ROS. ROS could induce neuronal damage as a result of oxidative stress. The hypothesis about the relationship between oxidative stress and TD comes from two studies that demonstrated the increased formation of lipid peroxidation products in the CSF of patients with TD (Lohr *et al.*, 1990b; Tsai *et al.*, 1998). Moreover, several studies have shown that vitamin E, a free-radical scavenger, has a positive effect on TD symptoms (Adler *et al.*, 1998).

Among the oxidative-stress pathway-related enzymes, SOD is the first-line antioxidant defense enzyme that plays an essential role in preventing the cell damage induced by free radicals. In particular, manganese SOD (MnSOD) is an intramitochondrial enzyme that scavenges the superoxide anions produced by mitochondrial energy metabolism (Fridovich, 1974; Robinson, 1998). Several studies have investigated the association between the *MnSOD* gene and TD, some of which have produced positive findings (Galecki *et al.*, 2006a,b; Hitzeroth *et al.*, 2007; Hori *et al.*, 2000). In addition, a recent meta-analysis revealed a significant association between TD and the *MnSOD* gene (Bakker *et al.*, 2008). However, these studies have provided conflicting data as to which genotype and allele are associated with TD, and most were based on small samples of TD subjects in genetic association studies. Two Korean studies, including ours, did not find a significant association between TD and the Ala-9Val SNP of the *MnSOD* gene (Kang *et al.*, 2008b; Pae *et al.*, 2007); however, we did show that this SNP is related to the severity of TD (Kang *et al.*, 2008b).

GST is also involved in the oxidative stress system. This enzyme is one of the important phase II drug-metabolizing enzymes that catalyze the conjugation of various endogenous and exogenous compounds, including antipsychotic drugs with reduced glutathione. de Leon *et al.* (2005b) reported an association between TD and the *GSTM1* polymorphism, but did not find an association between TD and *GSTT1*, *DRD2*, and P-glycoprotein (*MDR1*) polymorphisms. Al Hadithy *et al.* (2010) found that the *GSTP1* polymorphism is associated with TD. To further investigate the oxidative stress hypothesis of TD development, we analyzed whether genetic variants of *GSTM1*, *GSTT1*, and *GSTP1* are associated with neuroleptic-induced TD. We found that *GST* gene polymorphisms do not confer increased susceptibility to TD in patients with schizophrenia, but that TD severity might be related to *GSTP1* variants (Kang *et al.*, 2009). However, another Korean study was not able to confirm this association (Pae *et al.*, 2004a).

Studies examining the association between TD and other antioxidant genes such as the NAD(P)H quinone oxidoreductase gene (*NQO1*), and the genes for glutathione peroxidase and nitric oxide synthase (*NOS*) have produced inconsistent results. Many studies found no relationship between TD and these antioxidant enzyme genes (Hori *et al.*, 2006; Shinkai *et al.*, 2004, 2006; Zai *et al.*, 2010), while several others have found a significant association between the *NQO1* and *NOS3*

genes and TD (Liou *et al.*, 2006b; Pae *et al.*, 2004b). Thelma *et al.* (2007) performed a genetic association study to determine any links between variants of the several oxidative-stress-related genes (*SOD2*, *UCP2*, *NOS1*, *NOS3*, *GSTM1*, *GSTT1*, *GSTP1*, and *NQO1*) and the development of TD in Indian patients with schizophrenia, and found a tendency toward an association between the *NOS3* variant and the severity of TD. Table III summarized the studies reporting significant association between ROS-related genes and TD.

## V. Other Genes Reported to be Associated with TD

### A. Estrogen Receptor

It has been suggested that estrogen modulates dopamine receptors in the central nervous system and decreases the incidence and/or relieves the symptoms of TD. However, in a study of the relationship between estrogen receptor-α gene polymorphisms and TD in 118 schizophrenia and 128 matched non-TD schizophrenia patients, Lai *et al.* (2002) found only a marginal association.

### B. Opioid Receptor

There are several lines of evidence that the opioid receptors are involved in the pathology of TD (Cadet and Rothman, 1986; Sasaki *et al.*, 1996). Substance abuse, which increases vulnerability to TD in patients with schizophrenia (Dixon *et al.*, 1992), is associated with polymorphisms of the μ-opioid receptor gene polymorphism (Bond *et al.*, 1998). Ohmori *et al.* (2001) reported that the 118G allele of the μ-opioid receptor gene was significantly less common in those with TD.

### C. G-Protein Related Genes

Animal studies have found that abnormal involuntary movements develop in G-protein signaling gene knockout or mutant mice (Kovoor *et al.*, 2005; Rahman *et al.*, 2003), which suggests that the G-protein signal transduction process is associated with antipsychotic-induced TD. Therefore, variations in the regulator of G-protein signaling (*RGS9*) gene may be consequential for the development and/or severity of TD. A significant haplotypic association from *RGS9* gene was reported among TD subjects from Taiwan (Liou *et al.*, 2009). Our group investigated the functional SNP of another G-protein gene, G-protein $\beta 3$ subunit gene, with TD susceptibility in Korean patients with schizophrenia, but did not find any such association (Lee *et al.*, 2007b).

Table III
Studies Reported Significant Association Between Oxidative Stress–Related Genes and TD.

| Genes investigated | SNP (genotype or allele) | Findings | Ethnicity | Investigators, year |
|---|---|---|---|---|
| ***MnSOD*** | Ala9Val | Ala9: protective against TD | Japanese | Hori *et al.*, 2000 |
| | Ala9Val | Val/Val genotype: association with TD | Polish | Galecki *et al.*, 2006 |
| | Ala9Val | The Ala/Ala genotype had significantly lower AIMS scores | Xhosa | Hitzeroth *et al.*, 2007 |
| | Ala9Val | Ala allele carriers: more severe abnormal movements | Korean | Kang *et al.*, 2008b |
| | Ala9Val of *MnSOD* and others | Ala-Val genotype and Val allele: protective against TD | Meta-analysis | Bakker *et al.*, 2008 |
| ***GSTM1*** | Null/Wild | *GSTM1* Null: association with TD | Mixed ethnicity | de Leon *et al.*, 2005 |
| ***GSTM1,T1,P1*** | Ile105Val of *GSTP1* | Ile/Ile genotype of *GSTP1* had higher AIMS score | Korean | Kang *et al.*, 2009 |
| ***GSTP1, SOD2*, and *GPX1*** | Ile105Val of *GSTP1*<br>Ala-9Val of *MnSOD*<br>Pro197Leu of *GPX1* | 105Val allele of *GSTP1* associated with lower risk of TD | Russian | Al Hadithy *et al.*, 2010 |
| ***NQO1*** | 609C/T | T allele: associated with TD, T/T genotype: more severe TD | Korean | Pae *et al.*, 2004 |
| ***NOS3*** | -786T/C, 27 bp VNTR, Glu298Asp | T-4b-Glu haplotype: higher in non-TD | Chinese | Liou *et al.*, 2006b |
| ***SOD2, UCP2, NOS1, NOS3, GSTM1,T1,P1*, and *NQO1*** | *NOS3* 27 bp ins/del and other SNPs | Homozygote of *NOS3* 27 bp ins allele: more severe TD | Indian | Thelma *et al.*, 2007 |

TD, tardive dyskinesia; SNP, single-nucleotide polymorphism; MnSOD, manganese superoxide dismutase; AIMS, abnormal involuntary movement scale; DRD2, dopamine D2 receptor; CYP, cytochrome P450; COMT, catechol-*O*-methyltransferase; GST, glutathione-S-transferase; GPX, glutathione peroxidase; NQO, quinone oxidoreductase; NOS, nitric oxide synthase; VNTR, variable number of tandem repeat; UCP, uncoupling protein; bp, base pair.

### D. BDNF

Neurodegenerative processes may be involved in the pathogenesis of TD (Lohr *et al.*, 2003). BDNF has been regarded to play a critical role in the maintenance of functional neurons (Lewin and Barde, 1996). Tan *et al.* (2005) reported that serum BDNF levels were lower in patients with TD than in non-TD patients, and that the levels in the TD patients were inversely correlated with their AIMS score, suggesting that BDNF plays an important role in the pathophysiology of TD. However, other studies have found no such association between *BDNF* gene variants and TD (Kang *et al.*, 2008c; Liou *et al.*, 2004b; Park *et al.*, 2009; Wang *et al.*, 2010; Zai *et al.*, 2009b). A recent Korean study found significant gene–gene interactions between *BDNF* and glycogen synthase kinase 3 gene variants on TD susceptibility (Park *et al.*, 2009).

### E. Melatonin Receptor

Melatonin receptor genes (*MTNR1A* and *MTNR1B*) were studied very recently to ascertain their relationship to TD susceptibility because of their modulating effect on dopaminergic neurotransmission in the brain (Lai *et al.*, 2010). In relatively large Taiwan samples (256 TD patients and 162 non-TD subjects), that study revealed that *MTNR1A* haplotypes are associated with TD.

## VI. The Genome-Wide Association Approach

The genetic study method that is most widely used and provides an ample number of samples is the case-control association study. In practice, because TD is a side effect that only develops in patients with schizophrenia who are taking antipsychotics, it is difficult to conduct a linkage study involving family members with TD. For this reason, the hypothesis-based association study with the candidate gene approach is thought to be one of the best methods of TD research, providing substantial statistical power. The hypothesis-based approach toward candidate gene selection is attempted based on their relevance to the pharmacologic actions (pharmacokinetics and pharmacodynamics) of the drug and their relevance to the etiology and pathogenesis of the phenotype being investigated. However, since it is widely accepted that TD is caused by multiple genes, it is recognized that minor effects of several genes are responsible for the development of TD. A growing number of studies are focusing on gene–gene and gene–environmental-factor interactions. Even in cases where individual genes show no association with the occurrence of TD, interactions between several genes have been reported to be

related to the development TD in several cases. GWASs involve the examination of all or most of the genes (the genome) in different individuals of a particular species to see how much they vary between individuals. GWASs are necessarily hypothesis-free, and thus the entire genome is searched for associations rather than focusing on small candidate areas.

GWASs have recently been introduced to studies of TD, several of which have now been published. An unexpected association has been found between TD and SNPs, which have not previously been considered as candidate genes (Aberg *et al.*, 2010; Greenbaum *et al.*, 2010; Inada *et al.*, 2008; Syu *et al.*, 2010). The genes or polymorphisms reported to be associated with TD through these recent studies are rs7669317, located 167 kb from pyrophosphatase (inorganic) 2 (*PPA2*) and 16 kb from the locus encoding the hypothetical protein FLJ20184 on chromosome 4q24 (Aberg *et al.*, 2010), GLI family zinc finger 2 (*GLI2*) gene (Greenbaum *et al.*, 2010), GABA pathway genes (Inada *et al.*, 2008), and the heparan sulfate proteoglycan 2 (*HSPG2*) gene (Syu *et al.*, 2010). Therefore, this new technology may help to define novel candidate genes or pathways, improve our understanding of the pathogenesis of disease, and elucidate a spectrum of new potential drug targets. However, thus far their findings have not been consistent, which may be attributable to small samples and population stratification. GWASs usually require >1000 cases and the same number of controls; however, previous GWASs for TD have involved cohorts that were too small (<150). The differences in allele frequency relative to ethnicity are crucial in genetic studies. Ancestry-informative markers, a method that simplifies the ethnicity factor in research, or categorizing research results according to each ethnic group are necessary. Table IV summarized the other genes associated with TD in candidate gene studies and GWASs.

## VII. Future Research: Copy-number Variations and Epigenetics

Other new genetic testing technologies have recently been developed. Some researchers have stressed that other types of genetic variation such as deletions or duplications—the so-called copy-number variations (CNVs)—may have been neglected (Redon *et al.*, 2006). Moreover, other less common genetic variations such as microsatellite polymorphisms and translocations, inversions, and substitutions may be relevant to pharmacogenomics (Court, 2007). Unfortunately, many of the current platforms and systems used for genotyping do not pay much attention to CNVs (Ouahchi *et al.*, 2006) and were first discovered in *CYP2D6* (Ingelman-Sundberg *et al.*, 2007), an important pharmacogenetic gene. Autism (Sebat *et al.*, 2007) and schizophrenia (Walsh *et al.*, 2008) have been reported to be associated with CNVs. However, it is not known whether CNVs are relevant to TD susceptibility.

Table IV
Studies Reported Significant Association of Other Genes with TD Including GWASs.

| Genes investigated | SNP (genotype or allele) | Findings | Ethnicity | Investigators, year |
|---|---|---|---|---|
| Mu and delta Opioid receptor | 118A/G of mu<br>921T/C of delta | 118G allele: less frequent in TD | Japanese | Ohmori *et al.*, 2001 |
| *ESR1* | PVUII and Xbal | PvuII polymorphism: marginal association with TD | Chinese | Lai *et al.*, 2002 |
| BDNF and GSK3beta | Val66Met of BDNF<br>507T/C of GSK3beta | CC homozygote of GSK3beta with Val allele of BDNF: lower risk of TD | Korean | Park *et al.*, 2009 |
| *RGS9* | 7 SNPs | AGG haplotype (rs8077696-rs8070231-rs2292593): association with TD | Taiwan | Liou *et al.*, 2009 |
| *MTNR1A and MTNR1B* | 6 SNPs | Significant association between the haplotype ATG in the *MTNR1A* gene and non-TD | Taiwan | Lai *et al.*, 2010 |
| *SLC6A11, GABRB2, and GABRG3* | 3 SNPs | rs4684742 of *SLC6A11* associated with TD | Korean | Lee *et al.*, 2011 |
| Partial GWAS | 40573 SNP | GABA pathway genes *(ABAT, ALDH9A1, GABRA3, GABRA4, GABRB2, GABRAG3, GPHN,* and *SLC6A11)* associated with TD | Japanese | Inada *et al.*, 2008 |
| GWAS | 492000 SNP | rs7669317: associated with AIMS score | CATIE study sample (mixed ethnicity) | Aberg *et al.*, 2010 |
| GWAS | 495000 SNPs | rs3943552 T allele in the *GLI2* gene: associated with TD in Ashkenazi subsample | CATIE study sample (mixed ethnicity) | Greenbaum *et al.*, 2010 |
| Partial GWAS | 40573 SNPs → 24 SNPs in the HSPG2 gene | rs2445142 of HSPG2: associated with TD | Japanese | Syu *et al.*, 2010 |

TD, tardive dyskinesia; SNP, single-nucleotide polymorphism; ESR, estrogen receptor; BDNF, brain-derived neurotrophic receptor; GSK, glycogen synthase kinase; RGS, regulator of G protein signaling; MTNR, melatonin receptor; GABA, gamma-aminobutyric acid; SLC6A11, solute carrier family 6 member 11; GABRB2, GABA A receptor, beta 2; GABRG3, GABA A receptor, gamma 3; GWAS, genome-wide association study; ABAT, 4-aminobutyrate aminotransferase; aldehyde dehydrogenase 9 family, member A1; GABRA3, GABA A receptor, alpha 3; GABRA4, GABA A receptor, subunit alpha 4; GPHN, Gephyrin; AIMS, abnormal involuntary movement scale; CATIE, clinical antipsychotic trials of intervention effectiveness; GLI2, GLI family zinc finger 2; HSPG, heparan sulfate proteoglycan.

The importance of epigenetics in psychiatry (Abdolmaleky *et al.*, 2005) and psychotropic drug responses (Sharma *et al.*, 2006) is increasing. DNA methylation, histone modification, and micro-RNA interference are examples of epigenetics. Some epigenetic variants can be inherited by offspring, indicating the existence of a mechanism for biological heredity not based on the DNA sequence, a finding for which there is some evidence. For example, cigarette smoking by a grandmother increases the risk of asthma in the granddaughter (Li *et al.*, 2005). Famine in males in early puberty can exert an unknown epigenetic change in his grandson, resulting in a fourfold lower risk of type 2 diabetes; famine in the same population, among females while their oocytes are forming in utero, increases the risk of obesity and diabetes fourfold in that baby's granddaughter (Pembrey, 2002). In utero exposure of the mother to microbial-rich environments such as farms protects against the development of atopic sensitization and enhances the innate immune system in her children (Ege *et al.*, 2006).

There is accumulating evidence that epigenetic mechanisms are involved in various diseases as well as during normal development (Feinberg, 2007). However, the relevance of epigenetics in the pharmacogenetic response in humans is not well understood (Nebert *et al.*, 2008). Drug tolerance to an anesthetic in the fly has been shown to be caused by epigenetic histone modification and transcriptional induction (Wang *et al.*, 2007). Epigenetic effects increase with each passing decade of life, due to constant bombardment by environmental stimuli (including drugs and chemical or metal toxicants). Epigenetic alterations of response-related genes, such as dopamine and serotonin, have been suggested (Abdolmaleky *et al.*, 2005; Flomen *et al.*, 2004) and differences in DNA methylation in the regulatory regions of the *DRD2* and *COMT* genes have been identified (Petronis, 2006). It is currently unclear how epigenetic changes can be tested in the clinical setting, but it has been suggested that pyrosequencing is a technology that can be used for genetic changes including SNPs, CNVs, and methylation status (Marsh, 2007). Within the next several years, we expect to find examples of epigenetic-mediated effects on gene–drug interactions, especially on the development of TD development.

## VIII. TD as a Phenotype

While many significant findings of genetic studies for TD have been reported, the results obtained in a considerable proportion of these studies have not been replicated. The more important causes for these discrepancies include study design, sample size, and ethnicity. Diagnosing TD is not simple, and the diagnostic criteria and/or inclusion criteria for TD have differed somewhat among TD studies. Most of the pharmacogenetic studies of TD have adopted the AIMS (Guy, 1976) and/or criteria for TD proposed by Schooler and Kane (1982), which

requires the persistence of TD symptoms for 3 months for a TD diagnosis. However, some studies conclusively diagnosed TD after a single interview and physical examination, subsequently including the relevant patients as TD samples. Interpreting the results and making comparisons among the studies are thus limited due to the different inclusion criteria. A prospective study design minimizes this problem, but many studies are performed retrospectively for practical reasons.

Another important issue is the wide diversity of medications for patients with schizophrenia. Since individual antipsychotics exhibit various receptor profiles and pharmacological characteristics, the neurologic impacts may differ between patients. It is therefore necessary to control for antipsychotics. However, in clinical practice it is common to prescribe diverse and combined medications to schizophrenic patients and to use antipsychotics in polypharmacy regimens. In addition, since many other factors including age, gender, smoking, dosage, and duration of exposure to antipsychotics influence the development of TD, appropriate type of statistical analysis should be considered to control the demographic factors. In addition, separate analyses will be required as to the orofacial movement and the limb-truncal movement because it is thought that they are biologically different, depending on which abnormal movements are involved.

## IX. Conclusion

Relatively consistent findings have been reported on certain polymorphisms such as *CYP2D6**10, *DRD2* Taq1A, *DRD3* Ser9Gly, *HTR2A* T102C, *SLC6A11*, and *MnSOD* Ala9Val, although they remain controversial. Moreover, several GWASs on TD have found positive findings with rs7669317 on 4q24, *GLI2*, GABA pathway genes, and *HSPG2*. Although many genetic studies have been conducted on TD, the positive results of a considerable proportion of these studies have not been replicated or are inconsistent. These discrepancies seem to be attributable to differences in study design, sample size, and ethnicity. Furthermore, epigenetic approaches are also necessary and are expected to be associated with variations in antipsychotic response. These kinds of novel approaches to pharmacogenomic studies of TD will undoubtedly improve our knowledge about the determinants of the variability in antipsychotic-drug-induced TD and dyskinetic movement.

## Acknowledgment

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-313-E00333).

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# ANIMAL MODELS OF TARDIVE DYSKINESIA

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Tardive dyskinesia (TD) is a complex hyperkinetic syndrome characterized by choriform, athetoid, and rhythmic abnormal involuntary movements. Even though various neuroprotective strategies have been explored for the management of TD, nevertheless, the condition is difficult to treat. Various homologous, analogous, and correlational animal models have been standardized to understand the complex neurobiology of TD. The most common animal models include chronic administration of different typical neuroleptic agents to rodents that may lead to the development of (i) vacuous chewing movements, (ii) tongue protrusions, and/or (iii) facial jerking. The drug molecules that prevent or decrease the outcome of these symptoms are considered to be antidyskinetic agents. However, these animal models do not mimic the exact human condition and possess several phenomenological and methodological problems and therefore need clinical validation. The present review will discuss some of these animal models in context of exploring the novel drug targets in treating patients suffering from TD.

## I. Introduction

Tardive dyskinesia (TD) is a severe movement disorder characterized by involuntary movements of the tongue, lips, face, trunk, and extremities. These abnormal actions are commonly observed in patients who are chronically treated with typical neuroleptic agents. Some of the other agents such as anticholinergics, toxins, and substance of abuse may also induce TD symptoms. Various theories have been put forward to understand the neuropathology of TD. Some of these include

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00011-0




0074-7742/10 $35.00

dopaminergic hypersensitivity, disturbed balance between dopamine and cholinergic systems, dysfunctions of striatonigral GABAergic neurons, and excitotoxicity (Kulkarni and Naidu, 2003). One treatment strategy for these patients is to suspend the use of typical neuroleptic agent and instead prescribe atypical antipsychotics, mood stabilizers agents (lithium carbonate, carbamazepine, and divalproex sodium), benzodiazepines, or antidepressants. Unfortunately, the symptoms persist even after the withdrawal of typical antipsychotic drugs, indicating that these drugs produce some long term alterations in the brain tasks that are no longer associated with drug usage. Based on the above facts, it can be concluded that there are still numerous unanswered questions in the understanding of the pathophysiology of TD that requires step-by-step exploration. This will be possible only if there are suitable animal models mimicking the human disease condition.

Animal models have always been an important tool in understanding the complex pathophysiology of the diseases and in developing effective treatment strategies. Some of the basic requirements of animal models for studying neuropsychiatric disorders include symptom similarity, pharmacological isomorphism, and cross-species biochemical processes. These models should have face construct and predictive validity. Although these animal models never imitate the exact human disease condition, they allow for close approximation. For example, nonhuman primates due to their close proximity with human physiology are often considered as a more justifiable tool for unscrambling the anonymities of various human neurological disorders including TD. However, this animal species is difficult to manage and not cost-effective. Therefore, in the laboratories, rodents are generally employed due to their ease of handling, easy of breeding, cost, and resemblance with the human brain in many aspects. In this context, different animal models using both rodents and nonhuman primates have been standardized and validated to study the neuropathogenesis of TD and to explore various therapeutic strategies.

There are three general types of animal models that have contributed significantly in understanding the pathophysiology of TD (Kulkarni and Naidu, 2001a). These can be categorized as homologous, analogous, and correlational. The homologous models are the extremely predictive models of the clinical picture of TD and acquire similar etiology, biological basis, symptoms, response to treatment, course and outcome as well as unique features such as individual susceptibility. The long-term neuroleptic treatment with neuroleptics in nonhuman primates represents the homologous model of TD (Kulkarni and Naidu, 2001a). However, these animal models are very expensive and time-consuming to work. In contrast to homologous models, the analogous models have some of the critical features similar to that of TD (Kulkarni and Naidu, 2001a): for example, the VCMs observed in rodents when neuroleptic agents are administered for either short or extended period of time. These models in contrast to homologous models are efficient, inexpensive, and less time-consuming. While ideally a homology should be aimed for, isomorphic models may be useful for certain investigations.

The correlational models are somewhat different than homologous and analogous models. The correlational models require only few or no similarities between preclinical and clinical observations; however, the results of the preclinical model are highly predictive of the clinical picture (Kulkarni and Naidu, 2001a): for example, possible correlation between the likelihood of specific antipsychotic drugs to cause TD and acute extrapyramidal syndrome induced by neuroleptics or response to specific dopamine agonists following brief neuroleptic treatment in rodents. The correlational models are also efficient; however, the results are strain dependent and not highly predictive (Kulkarni and Naidu, 2001a).

An ideal animal model of TD should possess the following characteristics:

- Abnormal involuntary repetitive movements of the oral region that extends to extremities or trunk.
- Delayed development of these movements after chronic administration of neuroleptics and these movements persists even after the withdrawal of neuroleptic agent.
- Symptoms antagonized by dopamine receptor antagonists.
- Symptoms aggravated with stress.
- Symptoms resistant to suppression with anticholinergic agents.

The two analogous animal models that are generally employed to study the neuropathogenesis of TD include haloperidol- and reserpine-induced TD in rodents (mice and rats). Haloperidol, a $D_2$ dopamine receptor blocker, is a potent antipsychotic agent that is known to block the action of dopamine on its receptors. Chronic dopamine receptor blockage may lead to the supersensitivity of dopamine receptors and produce TD- like symptoms. Reserpine, on the other hand, is a neurotransmitter depleting agent that decreases the levels of norepinephrine, serotonin, and dopamine in the brain. Although, reserpine is not classified as a typical neuroleptic agent, it is being prescribed as an antipsychotic agent in some rare instances. Unfortunately, reserpine is also associated with the development of TD problem. Reserpine at low doses is known to induce orofocial movements late during the course of administration and these movements persist for a long time even after cessation of its administration.

The symptoms of TD that are generally observed in neuroleptic or reserpine-induced TD in rodents include (i) vacuous chewing movements (VCMs) referred to as single mouth openings in the vertical plane not directed toward physical material. The term oral dyskinesia was referred by Glassman and Glassman to refer to VCMs characterized by up and down jaw movements with occasional tongue protrusions (TP) (Glassman and Glassman, 1980). Rupinak and colleagues used the term "perioral movements" describing the purposeless chewing jaw movements with occasional TP with no wide openings. The VCMs observed are independent of and evident physical material. However, in contrast to actual TD symptoms that are observed in humans, the VCMs in rodents are generally

confined to the oral region and do not spread to the whole body, (ii) number of TP defined as stereotypically turning movements of the tongue with protrusions or fly catching tongue (iii) facial twitching.

Generally, on the test day, animals (rodents) are placed individually in a small plexiglas cage for the assessment of oral dyskinesia. Animals are generally acclimatized for at least 10 min before the start of counting. Hand-operated counters are used to score tongue protrusion and VCMs. Counting should be stopped whenever the animal starts grooming. Mirrors are always placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal faces away from the observer. Some of the other neuroleptics such as chlorpromazine and fluphenazine are also known to induce TD-like symptoms in rodents. The present review attempts to discuss some of the important animal models of TD and their significance in drug discovery. Haloperidol- and reserpine-induced TD are discussed in detail.

1. *Haloperidol-induced TD*

In one of the case reports published in 1980, a 7-year-old boy suffering from multiple tic or Gilles de la Tourette's disease was put on haloperidol therapy (Mizrahi *et al.*, 1980). Chronic administration of haloperidol in this child for 5 months resulted in the development of lingual-buccal-facial movements, a characteristic of TD (Mizrahi *et al.*, 1980). The dopamine $D_2$ receptor blocking property of haloperidol has been manipulated by the researchers to create an animal model of TD. Haloperidol (1–1.5 mg/kg) if administered chronically for approximately 21 days in rodents has been shown to produce TD-like symptoms (Naidu *et al.*, 2003a; Bishnoi *et al.*, 2008a). The symptoms are more severe in aged rats as demonstrated by significant increase in hyperkinetic motor activities, VCMs, TP, facial jerking, and development of dopamine supersensitivity (increased locomotor activity and stereotypy) as compared to young controls (Bishnoi *et al.*, 2007a). The mechanism of haloperidol-induced TD is not fully clear; however, various theories have been proposed. These include the following:

(i) *Dopamine receptor supersensitivity:* Chronic blockage of dopamine receptors by haloperidol in animals may lead to an upregulation of dopamine receptors and increased their sensitivity toward dopmine or dopaminergic agonists.
(ii) *Enhanced dopamine metabolism:* Chronic blockage of dopamine receptors may also result in an increase in metabolism of dopamine following its release. An increase in dopamine metabolism may lead to the generation of free radicals and thus enhance oxidative stress.
(iii) *Free radical generation:* Generation of excessive free radicals and oxidative stress by chronic haloperidol administration may damage different neurons such as GABAergic or dopaminergic systems and produce TD-like symptoms in animals.

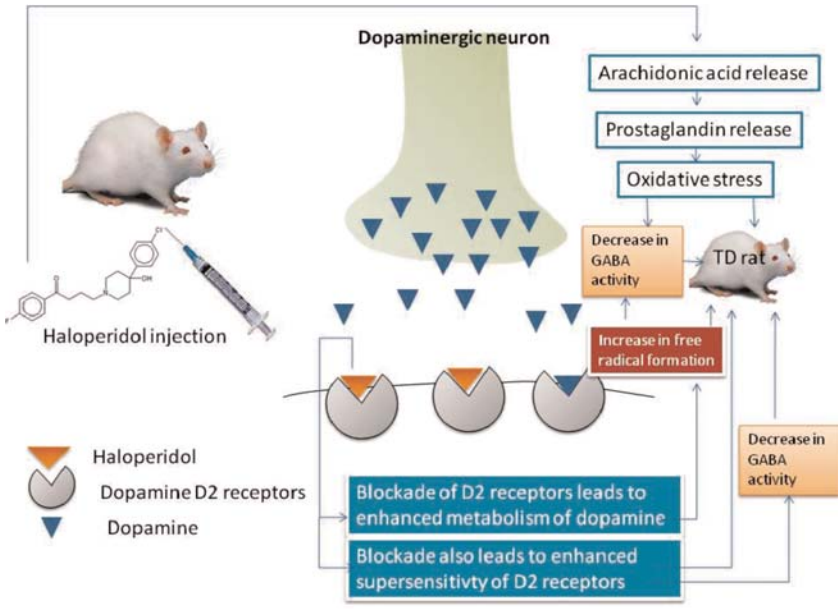


FIG. 1. Proposed mechanisms in haloperidol-induced TD in rats. In brief, chronic administration of haloperidol is known to block dopamine D2 receptors, which, in turn, can enhance the dopamine metabolism leading to formation of excessive free radicals. Chronic blockade of $D_2$ receptors may lead to enhanced supersensitivity of dopamine receptors. Furthermore, chronic administration of haloperidol may mobilize arachidonic acid, which can produce various inflammatory prostaglandins and cause neuronal cell death. All these changes induced by chronic administration of haloperidol may result in TD-like symptoms. (For color version of this figure, the reader is referred to the web version of this book.)

(iv) *Enhanced prostaglandin production:* Chronic haloperidol treatment may lead to enhanced production of inflammatory prostaglandins that, in turn, can increase the oxidative stress in the brain and exaggerate TD-like symptoms. All these proposed mechanisms have been illustrated in Fig. 1.

Chronic administration of haloperidol for 21 days in rodents has shown to produce VCMs, TP, and facial jerking. Neurochemical analysis have revealed that chronic administration of haloperidol (1 mg/kg for 21 days) reduces the levels of norepinephrine, dopamine, and serotonin in the striatum region of the rat brain when identified through *in vivo* microdialysis studies (Kulkarni *et al.*, 2009). Chronic administration of haloperidol has been associated with an increased expression of inflammatory markers such as TNF-α (tumor necrosis factor-alpha) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) (Post *et al.*, 1998; Post et al., 2002) that may lead to neurotoxicity. A study conducted in our laboratory has shown that chronic administration of haloperidol significantly increased the levels of TNF-α and NFkappaB p65 subunit in rat striatum

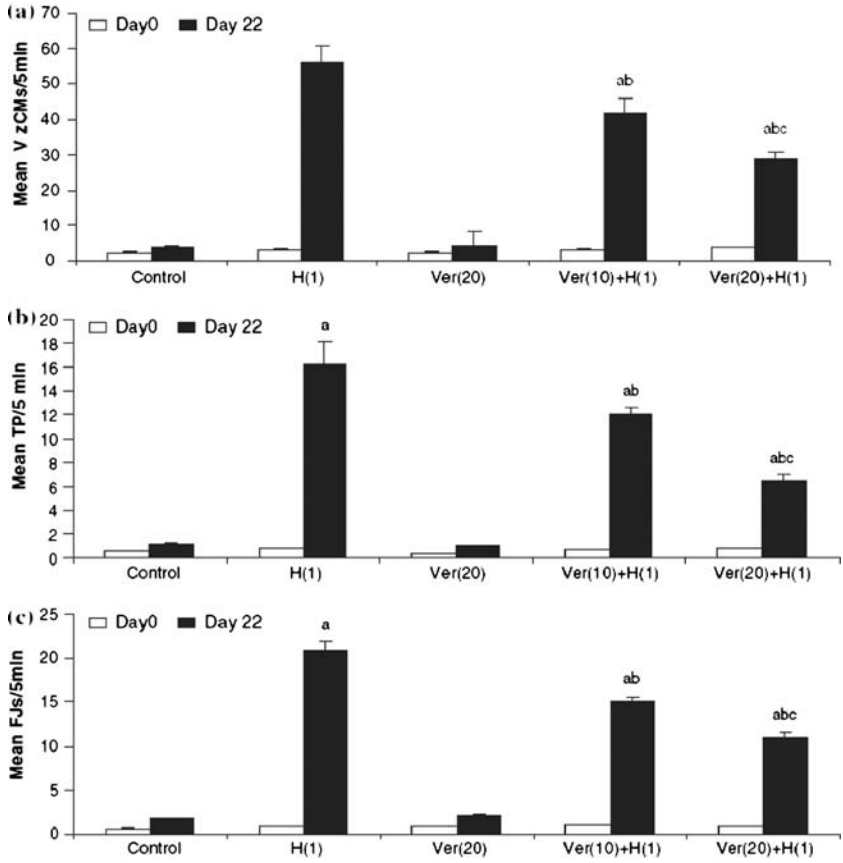


FIG. 2. (a) Vacuous chewing movements (VCMs), (b) TP, (c) number of facial jerking, recorded on day 0 and day 22 (test day) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), Verapamil (20 mg/kg), and Verapamil (10 and 20 mg/kg) + Haloperidol (1). Data are expressed in Mean ± SEM. $^{a}P < 0.05$ as compared to control group, $^{b}P < 0.05$ as compared to haloperidol group, and $^{c}P < 0.05$ as compared to verapamil (10) + haloperidol (1) group. H-Haloperidol; Ver-Verapamil. (Reproduced with permission from Bishnoi *et al.*, 2008c).

(Bishnoi *et al.*, 2008b). Various neuroprotective agents have been explored against haloperidol-induced orofacial dyskinesia. Some of these include:

*1a Calcium channel blockers:* The protective effects of calcium channel blockers have been studied in haloperidol-induced TD in rodents (Bishnoi *et al.*, 2008c). It has been found that administration of verapamil, diltiazem (both nondihydropyridines), or nifedipine (dihydropyridine) protected the animals against haloperidol-induced TD. These drugs attenuated the chronic haloperidol-induced VCMs, TP, and facial jerking (Figs. 2–4). Out of all the calcium channel blockers tested in this

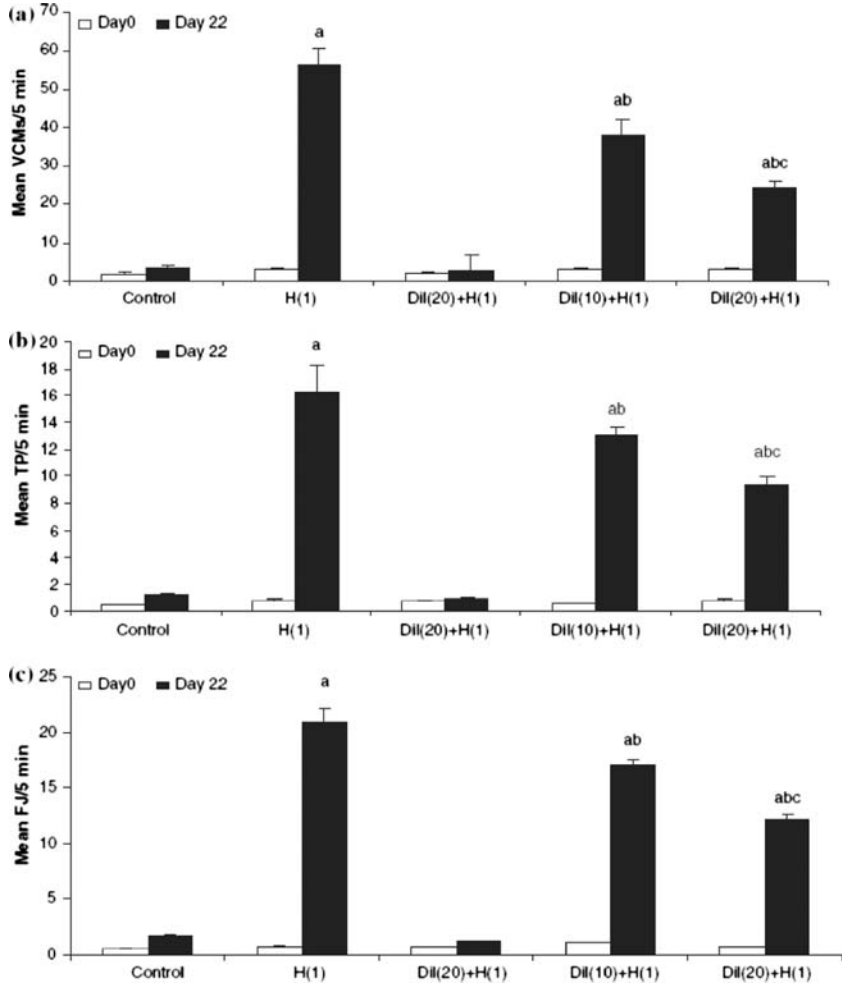


FIG. 3. (a) Vacuous chewing movements (VCMs), (b) TP, (c) number of facial jerking, recorded on day 0 and day 22 (test day) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), diltiazem (20 mg/kg), and diltiazem (10 and 20 mg/kg) + Haloperidol (1). Data are expressed in mean ± SEM. [a]$P < 0.05$ as compared to control group, [b]$P < 0.05$ as compared to haloperidol group, and [c]$P < 0.05$ as compared to diltiazem (10) + haloperidol (1) group. H-Haloperidol; Dil-Diltiazem. (Reproduced with permission from Bishnoi *et al.*, 2008c).

study, nifedipine was found to be the most effective (Bishnoi *et al.*, 2008c). Impaired cognitive function is a side effect of chronic neuroleptic administration. In this study, administration of haloperidol produced significant memory loss that was prevented by chronic treatment with calcium channel blockers. Following

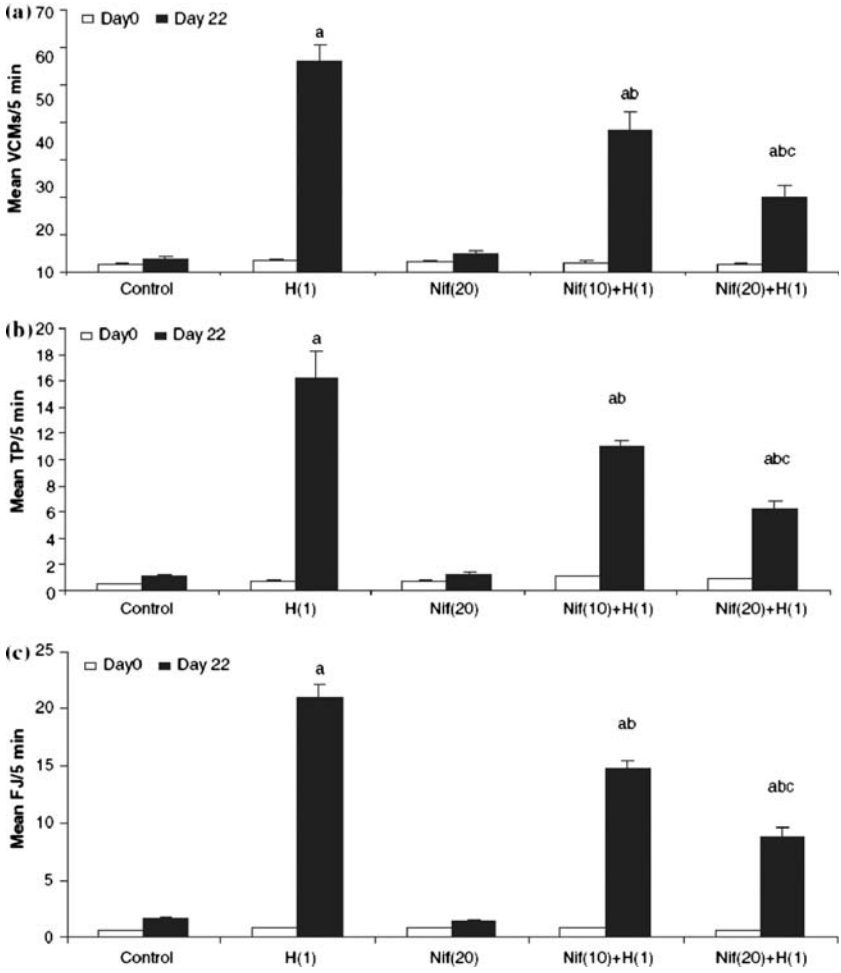


FIG. 4. (a) Vacuous chewing movements (VCMs), (b) TP, (c) number of facial jerking, recorded on day 0 and day 22 (test day) in rats chronically treated with vehicle, haloperidol (1 mg/kg, i.p. 21 days), nifedipine (20 mg/kg), and nifedipine (10 and 20 mg/ kg) + Haloperidol (1). Data are expressed in mean ± SEM. $^{a}P < 0.05$ as compared to control group, $^{b}P < 0.05$ as compared to haloperidol group, and $^{c}P < 0.05$ as compared to nifedipine (10) + haloperidol (1) group. H-Haloperidol; Nif-Nifedipine. (Reproduced with permission from Bishnoi *et al.*, 2008c).

behavioral observations, animals were sacrificed and brains were removed. The striatum was then isolated and analyzed for various oxidative stress parameters. It was found that chronic haloperidol resulted in an increase in levels of lipid peroxidation, superoxide anion generation, and significant decrease in the nonprotein

thiols and antioxidant enzyme pool (catalase and superoxide dismutase) (Bishnoi *et al.*, 2008c). Furthermore, neurochemical analysis has revealed that chronic administration of haloperidol decreases the levels of serotonin, dopamine, homovanillic acid, and hydroxyindole acetic acid in rat brain striatum homogenates that were reversed by the calcium channel blockers (Bishnoi *et al.*, 2008c). These preclinical findings are in agreement with clinical outcomes where calcium channel blockers particularly nifedipine has been shown to improve the TD symptoms in patients (Kushnir and Ratner, 1989).

*1b Neurosteroids:* Neurosteroids are the steroid molecules that are synthesized by the brain and nervous system and play an important role in various physiological functions of the body. They have been found to be protective in different disorders of the central nervous system such as epilepsy, traumatic brain injury, anxiety and depression, insomnia, etc. Some of the important neurosteroids include progesterone, dehydroepiandrosterone sulfate (DHEAS), pregnenolone sulfate (PS), allopregnanolone, etc. These neurosteroid molecules are known to act on different receptor systems, ion channels, and various signaling pathways in the brain. Some of these neurosteroids are positive allosteric modulators of $GABA_A$ receptors and thus speculated to play an important role in the pathogenesis of TD. This prompted us to explore the effect of various neurosteroids in animal models of TD. We examined the effect of progesterone (a positive modulator of $GABA_A$ receptor) against haloperidol-induced TD in rats (Bishnoi *et al.*, 2008d). Results revealed that chronic administration of progesterone (5–20 mg/kg, i.p.) dose dependently reversed the orofacial dyskinetic movements induced by chronic administration of haloperidol (Bishnoi *et al.*, 2008d). Progesterone also prevented the increase in lipid peroxidation and superoxide anion generation and the decrease in nonprotein thiols, catalase, and superoxide dysmutase induced by haloperidol. When neurochemical analysis was carried out in the striatum region of the rat brain, it was found that daily administration of progesterone attenuated decreases in dopamine levels induced by chronic administration of haloperidol (Bishnoi *et al.*, 2008d). Finasteride, a 5-alpha reductase inhibitor, prevented the protective effect of progesterone in the haloperidol-induced TD model (Bishnoi *et al.*, 2008d). Therefore, it was concluded that the protective effect of progesterone in this animal model is due to its metabolite, allopregnanolone (Bishnoi *et al.*, 2008d). In another study, various other neurosteroid agents were evaluated in the haloperidol-induced TD model. Again, allopregnanolone and progesterone attenuated the manifestation of haloperidol-induced orofacial dyskinesia whereas pregnenolone and DHEAS aggravated effect in this animal model (Bishnoi *et al.*, 2008a). It is important to mention here that allopregnanolone and progesterone are positive allosteric modulators of $GABA_A$ receptors while pregnenolone and DHEAS are negative modulators. Therefore, it can be concluded that haloperidol-induced VCMs and related behaviors are caused due to the decrease in GABAergic function, an increase in glutamatergic neurotransmission, and positive

GABA-modulating/negative NMDA-modulating agents can prevent the symptoms (Bishnoi *et al.*, 2008a).

*1c Lazaroids (21-aminosteroids):* These are 21-aminosteroid compounds that have demonstrated protective effect in various animal models of neurological disorders. Lazaroids have an antioxidant property through the stabilization of the cell membrane as well as maintain the membrane-stabilizing effect of glucocorticoids without the receptor-dependent side effects (Kavanagh and Kam, 2001). Lazaroids are nonglucocorticoid analogs of *methylprednisolone*. Methylprednisolone is neuroprotective when administered at higher doses and this protection is independent of its glucocorticoid activity (Kavanagh and Kam, 2001). In one of the studies carried out in our laboratory, we have explored the protective effect of lazaroid, U-74500A [pregna-1,4,9(11)-triene-3,20-dione, 21-(4-(5,6-bis(diethylamino)-2-pyridinyl)-1-piperazinyl)-16-ethyl-HCl (16-alpha)] in haloperidol-induced TD (Bishnoi *et al.*, 2007b). Our study revealed that chronic treatment of U-74500A (1–5 mg/kg., i.p.) attenuated the haloperidol-induced VCMs and related behavioral abnormalities (Bishnoi *et al.*, 2007b). Furthermore, U-74500A attenuated increased malondialdehyde levels (indicator of lipid peroxidation) induced by chronic administration of haloperidol in the cortex and striatum but not in the subcortical region of the rat brain (Bishnoi *et al.*, 2007b). All the above findings have suggested that this class of drug may be useful in treating patients suffering from TD. However, proper clinical evidence is required before we may consider the use of lazaroids in therapeutics.

*1d N-methyl-d-aspartate (NMDA) receptor antagonists:* Chronic blockage of dopamine $D_2$ receptors localized on glutamatergic terminals in the striatum region has been hypothesized to enhance the release of glutamate. This can, in turn, destroy the striatal neurons (Naidu and Kulkarni, 2001a). Therefore, NMDA receptor antagonists can be protective in treating patients suffering from TD. In preclinical findings, memantine, an NMDA receptor blocker, was shown to be neuroprotective in preventing behavioral alterations induced by chronic administration of haloperidol in rats. These animals were pretreated with memantine at doses of 20 and 40 mg/kg before being subjected to haloperidol injections. The experiment lasted for total of 20 weeks (Andreassen *et al.*, 1996). Additional studies demonstrated that glutamate upregulates striatal enkephalin levels that, in turn, play an important role in the development of haloperidol-induced persistent oral dyskinesias. Memantine, by decreasing the expression of preproenkephalin mRNA, is protective in haloperidol-induced TD (Andreassen *et al.*, 2003). Our lab has shown that dizocilpine, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, reversed the haloperidol-induced VCMs (Naidu and Kulkarni, 2001a). Similarly, antiexcitotoxic GM1 ganglioside has been found to be protective in this animal model (Andreassen and Jørgensen, 1994). Also, chronic treatment of haloperidol has been shown to induce the expression of cFOS (a cellular protooncogene belonging to the immediate early *gene* family of transcription factors) and

the expression of cFOS is significantly attenuated by pretreatment with MK-801, a noncompetitive NMDA receptor antagonist (Lee and Rajakumar, 2003). In a study, amantadine (a low-affinity, uncompetitive NMDA-receptor antagonist), ifenprodil (a noncompetitive allosteric NMDA receptor antagonist acting at the polyamine site), and Ro 25-6981 (a potent and selective blocker of NMDA receptors which contain the NR2B subunit) has been found to be effective in reversing haloperidol-induced orofacial dyskinesia (Konitsiotis *et al.*, 2006). The study has found that out of all these molecules, Ro 25-6981 is most effective in this animal model indicating that antagonists selective for NMDA receptors containing the NR2B subunit are more potent in reversing TD (Konitsiotis *et al.*, 2006).

*1e GABAergic agents:* Enhancing the GABAergic neurotransmission has been found to be protective in animal models of TD. In one of the studies, tiagabine, an indirect acting GABA agonist, reversed the haloperidol-induced TD (Gao *et al.*, 1994). Progabide, an analog and prodrug of gamma-aminobutyric acid, when administered in food for a total of 12 months prevented haloperidol-induced TD in rats (Kaneda *et al.*, 1992). Interestingly, progabide decreased the VCMs up to 40% as compared to its vehicle control group. These studies have suggested that GABAergic modulators can be potent antidyskinetic drugs (Kaneda *et al.*, 1992).

*1f Serotoninergic modulators:* Serotonin has an important role to play in the pathophysiology of TD and various serotoninergic modulators are known to be protective in these animal models. It has been shown by Kozell and colleagues that there is possible altered serotoninergic modulation in neuroleptic-induced dyskinesia and L-tryptophan (a serotonin precursor) was found to be protective in haloperidol-induced TD in rats (Kozell *et al.*, 1987). The 5-$HT_{1A}$ serotonin receptor agonists are known to protect animals against haloperidol-induced TD. It has been concluded that increasing the effect of somatodendritic 5-$HT_{1A}$ receptors by administering various serotonin receptor agonists can reduce the inhibitory influence of serotonin on the action of dopaminergic neurons to produce VCMs (Samad *et al.*, 2007). In this context, buspirone, a 5 $HT_{1A}$ receptor agonist has shown to be neuroprotective in animal models of TD (Haleem *et al.*, 2007). Similar to 5$HT_{1A}$ receptors, 5-$HT_{2A/2C}$ receptors are also known to play an important role in the pathophysiology of TD (Naidu and Kulkarni, 2001b). It has been demonstrated in our laboratory that both acute and chronic administration of seganserin, ketanserin, and ritanserin, 5-$HT_{2A/2C}$ receptor antagonists, reduce haloperidol-induced VCMs in a dose-dependent (0.05, 0.1, and 0.2 mg/kg, i.p.) fashion (Naidu and Kulkarni, 2001b).

*1g curcumin:* Researchers have established the protective effect of curcumin in almost all the disorders of the body. It is one of the common ingredients of Indian Curry and possesses anti-inflammatory and antioxidant properties. However, its use has been restricted in therapeutics due to very low oral bioavailability. There are different approaches proposed to enhance the bioavailability of curcumin. One

approach is to co-administer curcumin with piperine, a known inhibitor of hepatic and intestinal glucuronidation. As curcumin is known to possess antioxidant and anti-inflammatory properties, we hypothesized its protective effect in haloperidol-induced TD. Chronic administration of curcumin at doses of 25 and 50 mg/kg before haloperidol administration prevented the animals against haloperidol-induced behavioral, biochemical, and neurochemical alterations (Bishnoi *et al.*, 2008e). In this study, we also measured rearing (ability to stand) as one of the altered behavioral parameters induced by haloperidol administration (Bishnoi *et al.*, 2008e). Results demonstrated that chronic administration of haloperidol decreased the stereotypic rearing behavior up to seventh day that was thereafter increased up to last behavioral quantification (22 day). Pretreatment of curcumin prevented this increase of stereotypic rearing behavior (Bishnoi *et al.*, 2008e). Moreover, chronic haloperidol-challenged animals have demonstrated a decrease in cognitive ability when tested in elevated plus maze test and this deficiency was partially restored by chronic treatment with curcumin (Bishnoi *et al.*, 2008e). Neurochemical analysis has revealed that curcumin reversed the haloperidol-induced decrease in dopamine, norepinephrine, and serotonin in the homogenates of cortical and subcortical regions of rats. Based on the above findings, it can be concluded that the antidyskinetic effect of cucrumin might be due to its antioxidant or anti-inflammatory actions (Bishnoi *et al.*, 2008e).

*1h Zolpidem* [*N, N, 6-trimethyl-2-p-tolyl-imidazo (1, 2-a) pyridine 3-acetamide*]: Is a nonbenzodiazepine-related hypnotic drug with an imidazopyridine structure that binds to the omega site of the GABA-benzodiazepine chloride channel ionophore. The heterocyclic moiety present in zolpidem is hypothesized to be responsible for its antioxidant property. Similar kind of heterocyclic moiety is also present in melatonin, a powerful antioxidant. Like melatonin, zolpidem is also clinically used in subsiding jet-lag symptoms. Zolpidem is found to be protective in many neurological disorders. The neuroprotective effect of zolpidem is hypothesized to act through its GABA mimetic property and/or antioxidant effect. When tested in haloperidol-induced TD, zolpidem prevented the development of VCMs, TP, and facial jerking induced by chronic administration of haloperidol (Bishnoi *et al.*, 2007c). Biochemical analyses have revealed that chronic administration of haloperidol produced enhanced maliondialdehyde levels, significantly decreased nonprotein thiols (NPSH), as well as superoxide dysmutase and catalase activities in rat brain striatum that was reversed by pretreatment with zolpidem (Bishnoi *et al.*, 2007c). Additionally, chronic administration of haloperidol for 21 days enhanced the levels of vanillyl mandelic acid (VMA, metabolite of norepinephrine) and homovanillic acid (HVA, metabolite of dopamine) in urine. This effect was significantly reversed by chronic zolpidem treatment (Bishnoi *et al.*, 2007c).

*1i Adenosine reuptake inhibitors:* Adenosine is an inhibitory neurotransmitter in the brain and plays an important role in the pathophysiology of many neurological

disorders of the body including TD. Adenosine maintains a delicate balance between the GABAergic inhibitory and glutamatergic excitatory neurotransmission in the brain. Any kind of disturbance in this balance may lead to different neurological disorders. Our laboratory has tested the effect of dipyridamole, an adenosine reuptake inhibitor, and nimodipine, an adenosine transport inhibitor, in haloperidol-induced TD in rats (Bishnoi *et al.*, 2007d). Both dipyridamole and nimodipine were protective in haloperidol-induced TD (Bishnoi *et al.*, 2007d). Chronic administration of haloperidol decreased the turnover of dopamine and norepinephrine in both cortical and subcortical regions that was dose-dependently reversed by adenosine reuptake/transport inhibitors (Bishnoi *et al.*, 2007d). Moreover, another study has demonstrated the protective effect of caffeine ($A_{2A}$ receptor antagonist) when combined with adenosine in haloperidol-induced TD in rats. To this end, the combination of both adenosine and caffeine did not show any potentiating effect in this animal model. This may be due to the fact that both adenosine and caffeine are acting on the same receptor system (Bishnoi *et al.*, 2006). Theophylline, an $A_{2A}$ receptor antagonist, has also demonstrated protective action in this animal model (Bishnoi *et al.*, 2007e). All of the above-mentioned studies have demonstrated that adenosine neurotransmission plays a major role in the pathophysiology of TD and treatment with adenosinergic modulators may be beneficial for the patients suffering from TD. However, the efficacy/risk ratio should be considered carefully before these drugs are put into clinical use for the treatment of TD.

*1j Rutin:* It is a flavonol glycoside composed of a flavonol quercetin and disaccharide rutinose. Flavonoids possess multiple pharmacological activities in human body. Rutin is present in abundance in onions, apples, tea, and red wine. The antioxidant property of rutin may be responsible for its neuroprotective action. Rutin reversed haloperidol-induced VCMs, TP, and facial jerking when tested in rats (Bishnoi *et al.*, 2007f). The oxidative stress induced by chronic administration of haloperidol was reversed by chronic treatment with rutin. The study concluded with the fact that oxidative stress is involved in haloperidol-induced various behavioral and biochemical alterations in rats. Rutin due to its antioxidant potential is protective in this animal model (Bishnoi *et al.*, 2007f). Therefore, rutin may be considered as a future drug for the treatment of hyperkinetic movements in patients suffering from TD; however, the hypothesis still requires clinical evaluation and validation.

*1k Anti-inflammatory drugs:* Cyclooxygenase (COX) inhibitors are found to be protective in animal models of TD. COX is an enzyme that converts arachidonic acid released from membrane phospholipids into prostaglandins (PGs). COX enzyme exists in two isoforms, COX-1 and COX-2. COX-1 is also known as constitutive isoform of COX enzyme and known to be expressed in different organs of the body including stomach, kidney, bladder, and brain. In contrast, COX-2 is an inducible enzyme that is expressed in most of the tissues following any inflammation

or injury. Recent literature has indicated an important role of COX enzymes and prostaglandins in brain physiological functions. COX is expressed in different regions of brain that are important in the pathophysiology of TD. Our laboratory has explored the protective effect of different COX-inhibitors in animal models of TD (Naidu and Kulkarni, 2001c). Indomethacin, a nonselective COX-inhibitor is protective in haloperidol-induced TD. Indomethacin at dose range of 5–20 mg/kg reversed the VCMs induced by chronic haloperidol challenge (Naidu and Kulkarni, 2001c). These studies suggested an important role of prostaglandins in haloperidol-induced VCMs. Based on the above-mentioned studies, various anti-inflammatory drugs could be potential antidyskinetic drugs (Naidu and Kulkarni, 2001c). However, further clinical and preclinical findings are required to uncover the fact.

*1l: Other agents in haloperidol-induced TD:* Some of the other agents that have revealed protective action in haloperidol-induced TD include FK-506 (immunomodulatory drug) (Singh *et al.*, 2003), *Withania somnifera* (Naidu *et al.*, 2003a, 2003c), quercetin (Naidu *et al.*, 2003b), melatonin (Naidu *et al.*, 2003a), carvedilol (Naidu *et al.*, 2002), 5-$HT_3$ serotonin receptor antagonists (Naidu and Kulkarni, 2001d), alpha lipoic acid (Thaakur and Himabindhu, 2009), spirulina (Thaakur and Jyothi, 2007), ebselen (Burger *et al.*, 2005), SR48692 (neurotensin receptor antagonist) (McCormick and Stoessl, 2003), risperidone (Carvalho *et al.*, 2003), etc.

2. *Reserpine-induced TD*

Reserpine depletes various neurotransmitters and has been earlier prescribed to provide symptomatic relief in patients suffering from hypertension. However, the drug did not find much acceptance by the patients and clinicians due to its propensity to produce major depression in patients. The neurotransmitter-depleting property of reserpine has been manipulated by the researchers to develop an animal model for TD. The dopamine depleting property has been hypothesized to be responsible for reserpine propensity to induce TD symptoms. The reserpine-induce TD is faster in onset [(the effects are observed even after 5 days (every alternate day) of reserpine (1 mg/kg., s.c.) administration] as compared to other models. The reserpine-induced dyskinetic movements can last for at least 60 days postadministration. In contrast, the TD symptoms in humans generally develop after months or years of neuroleptic treatment.Various chemical moieties have been tested against reserpine-induced TD. These include the following:

*2a melatonin:* It is a powerful antioxidant. When tested in reserpine-induced TD, melatonin inhibited the occurrence of VCMs (Fig. 5). However, treatment of animals with luzindole or prazosin (antagonists for the putative melatonin receptors $MT_1/T_2$ and $MT_3$, respectively) or flumazenil (central benzodiazepine receptor antagonist) did not reverse the antidyskinetic effect of melatonin (Raghavendra *et al.*, 2001). However, PK11195, a peripheral

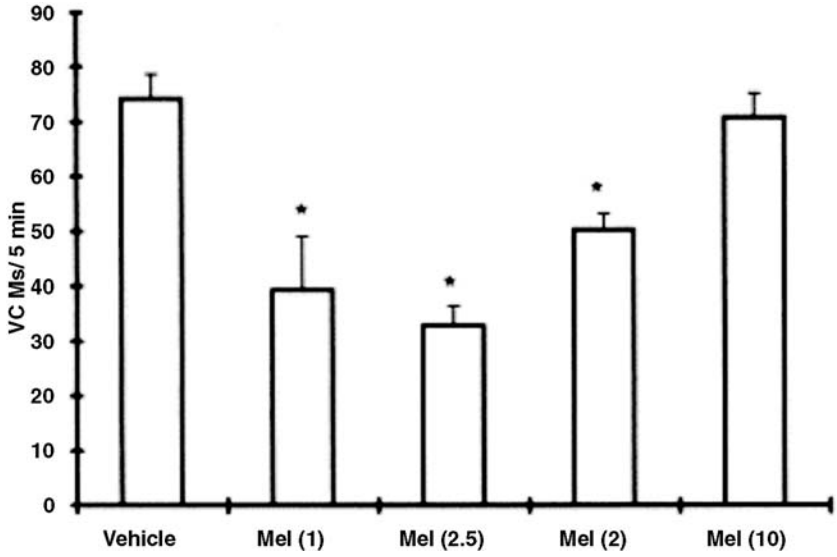


FIG. 5. Effect of melatonin (1–10 mg/kg, i.p.) against reserpine-induced vacuous chewing movements (VCMs) in rats. Values are expressed as mean ± S.D. of VCMs/5 min. ANOVA values $F(4,25) = 65.38$ ($P < 0.01$). *$P < 0.05$ compared to vehicle-treated group (Dunnett's *t*-test).
(Reproduced with permission from Raghavendra *et al.*, 2001).

benzodiazepine receptor antagonist, antagonized the melatonin reversal of reserpine-induced VCMs. This indicated that the antidyskinetic effect of melatonin involves peripheral but not central benzodiazepine receptors (Raghavendra *et al.*, 2001).

*2b quercetin:* Quercetin has been found to possess antidyskinetic activity in reserpine-induced TD. The protective effect of quercetin has been credited to its antioxidant potential. The following figure (Fig. 6) has demonstrated a decrease in VCMs and TP by quercetin in reserpine-induced TD animal model (Naidu *et al.*, 2004).

*2c Withania somnifera:* The root extract of *Withania somnifera* has been found to protect rodents from reserpine-induced TD. It was demonstrated that oxidative stress plays an important role in the pathophysiology of reserpine-induced VCMs and *Withania somnifera* due to its antioxidant property is protective in this animal model (Naidu *et al.*, 2006).

*2d valproic acid:* Valproic acid is an antiepileptic drug that acts through decreasing the sodium channels-induced neuronal depolarization. There is an enhanced excitotoxic reaction in the brains of TD patients. Therefore, administration of some powerful neuroprotective agent that can reduce the excitotoxic process in the brain may be a powerful antidyskinetic agent. One study has explored the action of

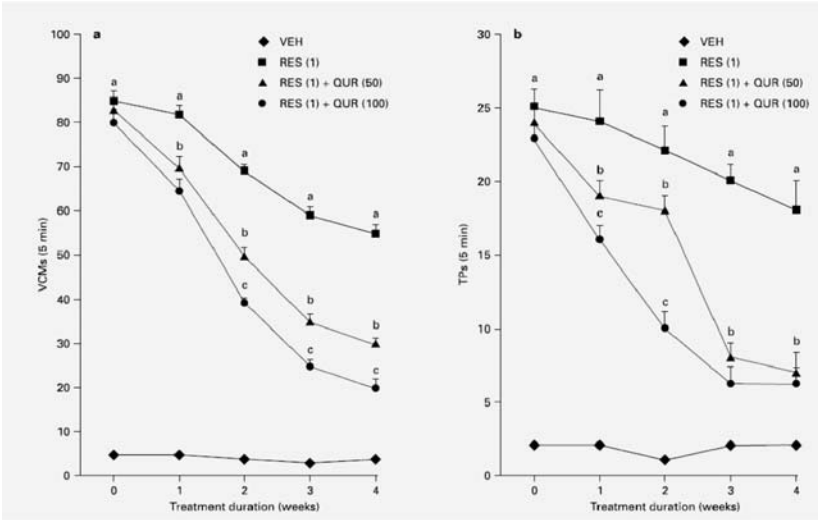


FIG. 6. Effect of chronic administration of quercetin (QUR) on reserpine (RES)-induced VCMs (**a**) and TPs (**b**) in rats. Values expressed as mean ± SEM. [a]$P < 0.05$ as compared with the vehicle (VEH)-treated control group. [b]$P < 0.05$ as compared with the reserpine- and quercetin 100 mg/kg-treated groups. [c]$P < 0.05$ as compared with the reserpine- and quercetin 50 mg/kg-treated groups (ANOVA followed by Tukey's test).
(Reproduced with permission from Naidu *et al.*, 2004).

valproic acid in rat model of TD. It has been found that valproic acid at dose of 200 mg/kg protected the rats from reserpine-induced orofacial dyskinesia (Peixoto *et al.*, 2003). This study strongly demonstrates the protective effect of valproic acid in ameliorating the symptoms of TD.

*2e GABAergic drugs:* It has been suggested that GABAergic hypofunction could be a consequence of neuroleptic-induced dopamine receptor supersensitivity and therefore GABAergic modulators could be protective in TD. Both tetrahydroisoxazolopyridine (THIP) ($GABA_A$ agonist) and baclofen ($GABA_B$ agonist) reversed reserpine-induced orofacial dyskinesia in rats (Peixoto *et al.*, 2005). However, diazepam (1-4 mg/kg) did not affect the manifestation of reserpine-induced TD. Similar kind of study has suggested the protective effect of baclofen in reserpine-induced TD (Castro *et al.*, 2006).

### 3. *Chlorpromazine-induced TD*

Chlorpromazine, the first and most famous drug introduced for the treatment of psychosis, revolutionized the treatment of psychiatric disorders. It is included under the category of aliphatic phenothiazines. However, chlorpromazine

Table I
Behavioral Effects of Chronic Haloperidol and Chlorpromazine Treatment in Rats. VCMs-Vacuous Chewing Movements.

| Treatment (mg/kg) | VCMs/5 min | Tongue protrusions/5 min |
|---|---|---|
| Vehicle | 6 ± 1 | 2 ± 0.577 |
| Haloperidol (1) | 58.667 ± 3.667 | 22.833 ± 1.167 |
| Chlorpromazine (5) | 52.833 ± 2.667 | 19.667 ± 2.167 |

has also been associated with the problem of TD. Our laboratory has standardized chlorpromazine-induced TD as an animal model to evaluate antidyskinetic drugs. Chlorpromazine when administer in a dose of 5 mg/kg to rats for a total of 21 days is known to produce orofacial hyperkinetic movements. The behavioral effects of both haloperidol and chlorpromazine-induced TD are compared in the following table (Table I). In brief, chlorpromazine at 5 mg/kg for 21 days produced similar numbers of VCMs and TP as compared to haloperidol (1 mg/kg for 21 days) in the rat model. *In vivo* microdialysis evaluation has demonstrated decrease in the levels of norepinephrine, dopamine, and serotonin in the striatal region of rat brain (Kulkarni *et al.*, 2009). Carvedilol (Naidu *et al.*, 2002), lazaroids (Bishnoi *et al.*, 2007b), neurosteroids (Bishnoi *et al.*, 2008d), and zolpidem (Bishnoi *et al.*, 2007c) have found to be protective in reserpine-induced TD.

4. *Isoniazid-induced TD*

Intracerbroventricular (i.c.v.) injection of isoniazid has been shown to produce TD symptoms in rats (Kulkarni and Naidu, 2001b). The probable mechanism of isoniazid-induced TD includes (i) depleting the GABAergic neurotransmission and (ii) abolishing the activity of glutamic acid decarboxylase (GAD). The symptoms of isoniazid-induced TD resembles very much with neuroleptic-induced TD in animal models (Kulkarni and Naidu, 2001b). Some of the similarities include the following:

1 VCMs induced by isoniazid are very much identical to those observed with chronic neuroleptic treatment in animal models (Kulkarni and Naidu, 2001b).
2 VCMs induced by isoniazid are significantly reversed by acute treatment with reserpine or haloperidol (Kulkarni and Naidu, 2001b). Acute treatment with reserpine or haloperidol has shown to reduce the neuroleptic-induced VCMs and reported to give symptomatic relief to the patients suffering from TD.

3 Isoniazid-induced vacuous chewing movements persisted for more than 30 days post-treatment. This is similar to that observed with neuroleptic-induced TD where these movements are known to persist for longer duration even after neuroleptic cessation sometimes is irreversible (Kulkarni and Naidu, 2001b).

## 5. *Primate model of TD*

The *Cebus apella* monkey is considered to be the most suitable animal model to study TD due to the fact that this species resembles human beings in many characteristics and also is very highly vulnerable to the effect of neuroleptic agents (Werge *et al.*, 2003). Chronic treatment of neuroleptics in monkeys (*Cebus apella*) can produce oral dyskinesia characterized by TP and facial grimacing (Bárány et al., 1983). Gunny and Barany have earlier measured the behavioral disturbances induced by chronic administration of haloperidol to *Cebus apella* monkeys (Gunne and Bárány, 1976). Haloperidol, a $D_2$ dopamine receptor blocker was administered chronically for 4–16 months once daily in the diet of the animals, and signs of acute dystonia–parkinsonian and buccolingual abnormal movements characterized of TD were noted (Gunne and Bárány, 1976). It was demonstrated that the chronic administration of haloperidol displayed the sign of sedation and Parkinson during first 5–7 weeks and later these animals developed acute dystonia (Gunne and Bárány, 1976), the symptoms characterized the human condition of chronic neuroleptic use. Furthermore, two of the monkeys developed the buccolingual signs characterized by grimacing and tongue protrusion after 3 and 12 months of chronic administration (Gunne and Bárány, 1976). In another study carried out on 11 *Cebus apella* monkeys, chronic administration of haloperidol (0.05–1.0 mg/kg/d) orally for up to 35 months resulted in the development of acute dystonic reaction (Bárány et al., 1979). Similar to that observed with haloperidol, fluphenazine enanthate, another antipsychotic agent, has also been shown to induce TD-like symptoms in monkeys (Kovacic and Domino, 1982). It has been demonstrated that biweekly injections of fluphenazine enanthate for 1 year may has led to the development of acute dystonia, dyskinetic, parkinsonian, and akathisia-like reactions in monkeys that get worsened after every injection and later on these animals developed the symptoms of TD similar to that observed in patients (Kovacic and Domino, 1982). The levels of various neuropeptides have shown to be altered in the monkey model of TD (Johansson *et al.*, 1990). The monkeys more susceptible to TD carry the gly9/gly9 DRD3 genotype (gene associated with TD in humans) (Werge *et al.*, 2003). However, in contrast to this, there was no ser23 $5HT_{2C}$ serotonin allele that has been reported to increase TD susceptibility in humans (Werge *et al.*, 2003). Various drug categories such as GABAergic or cholinergic modulators has shown to alleviate the symptoms of TD in this animal model (Báray and Gunne, 1979). The molecules like

Table II
Summary of Animal Models of TD.

| Animal model of TD | Treatment and evaluation of TD symptoms | Mechanism of TD |
|---|---|---|
| Haloperidol-induced TD | 1–1.5 mg/kg., i.p. for ~21 days and various parameters such as vacuous chewing movements (VCMs), TPs, and facial jerking are evaluated | • Chronic treatment with anti-dopaminergic agent may lead to the supersensitivity of dopamine $D_2$ receptors that, in turn, can produce TD-like symptoms<br>• Treatment with haloperidol may enhance dopamine metabolism which in turn can lead to enhance oxidative stress and thus death of dopaminergic or GABAergic neurons<br>• Chronic treatment with haloperidol may lead to enhance release of arachidonic acid and inflammatory prostaglandins |
| Reserpine-induced TD | 1 mg/kg., s.c. alternatively for 5 days and various parameters such as VCMs, TPs, and facial jerking are evaluated | • Reserpine depletes various monoamine levels such as norepinephrine, serotonin and dopamine<br>• It has been speculated that decrease in dopamine levels may lead to onset of TD<br>• It may enhance the oxidative stress in the brain |
| Chlorpromazine-induced TD | 5 mg/kg., i.p. for a total of 21 days and various parameters such as VCMs, TPs, and facial jerking are evaluated | • The mechanism is similar to haloperidol-induced TD |
| Isoniazid-induced TD | 1–10 mmol/rat., i.c.v. and vacuous chewing movements are measured. Peak effect can be observed after 30 min of its administration and effect may sustain for 60 min | • It depletes the GABA<br>• It abolishes the activity of glutamate decarboxylase |
| Neuroleptic administration in primate model of TD | Haloperidol (0.05–1.0 mg/kg/d) orally for up to 35 months and various parameters such as buccolingual signs characterized by grimacing and tongue protrusion are observed | • Dopamine receptor supersensitivity |

pramipexole (D3/D2 dopamine receptor agonist) and CIS-8-OH-PBZI ($D_3$ dopamine receptor agonist) reduced SKF 81297 ($D_1$ dopamine receptor agonist)-induced TD in Cebus monkeys (Peter *et al.*, 2004) demonstrating the role of dopamine receptors in the pathophysiology of TD.

## II. Limitations

Although, there are numerous animal models available to evaluate the pathophysiology and treatment strategies of TD, however, these models do not exactly imitate the human state of disease. To cite an example, the VCMs observed in animals are generally confined to the orofacial region. However, in humans these tremors may extend to other parts of the body such as face, neck, and limbs. Moreover, in animals, these VCMs are often noticeable after >2–3 weeks of subchronic haloperidol treatment that is relatively long time in the average life span of a rat (24 months). Also, besides the availability of these animal models, there is no clear-cut treatment available for TD. The GABAergic agents found effective in animal models of TD are sometimes not effective in clinics, the reason being unknown. Therefore, we need to explore more homologous animal models of TD that resembles the human disease in every specification.

## III. Conclusion

Various animal models of TD are summarized in Table II. Despite the presence of these enormous animal models, we are still not able to completely understand the pathophysiology of TD. However, these animal models have led the researchers to explore novel drug targets and validate the neurobiological concepts in understanding the TD. The animal models discussed above have their own advantages and disadvantages. There is a strong need to develop some of the new alternative animal models that has reasonable face and predictive validity and should be reproducible between investigators.

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# SURGERY FOR TARDIVE DYSKINESIA

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Tardive dyskinesia (TD) is an often bothersome side effect of antipsychotic treatment. Medical treatment options are usually disappointing. A few single case reports have suggested some efficacy of lesionning surgery (i.e. pallidotomy or thalamotomy). A much greater number of series (including one controlled-study) have assessed the effects of deep brain stimulation applied to the internal globus pallidus. All of them have shown a marked improvement of motor symptoms without any major psychiatric side effects.

## I. Introduction

Tardive dyskinesia (TD), consisting in a wide spectrum of abnormal involuntary movements (from dystonia to choreic-like movements), is an often bothersome side effect of a chronic antipsychotic treatment (for review, see Damier, 2009). For severe cases, medical treatment options are usually disappointing. The first option is to attempt withdrawing the antipsychotic drug if the psychiatric state of the patient permits it. However, in about half of the cases, TD persists even after more than a year without any intake of the incriminated drug. When the use of a neuroleptic is mandatory (e.g., for chronic psychotic diseases), a switch to an atypical neuroleptic such as clozapine is a useful option to consider. The risk of TD is indeed much lower with such a drug than with the classical antipsychotic


DOI: 10.1016/B978-0-12-381328-2.00012-2

0074-7742/10 $35.00

(Correll *et al.*, 2004). Several add-on treatments have also been proposed for reducing TD. Among them, tetrabenazine is probably the most effective but with the risk of inducing a depression or a parkinsonian syndrome (Kenney *et al.*, 2007). Many other drugs (i.e., vitamin E, calcium channel blockers, noradrenergic antagonists, and benzodiazepines) have also shown to have some efficacy (Soares and McGrath, 1999) In many patients however, despite all these attempts, distressing TD may persist and lead to a severe disability.

Since the late 1980s, there has been a renewed interest in surgery for treating various movement disorders, such as Parkinson's disease, tremor or dystonia. Such a treatment consists in a focal brain lesion or in continuous deep brain stimulation, the currently preferred option due to the reversibility (the stimulation can be turned off if required) and the adaptability (the electrical parameters can be fine-tuned in order to obtain the best ratio between effectiveness and stimulation-induced side effects) of the treatment. Three main targets are used for treating movement disorders: the subthalamic nucleus for Parkinson's disease (Krack *et al.*, 2003); the ventral intermediate nucleus of the thalamus for tremor (Benabid *et al.*, 1993); and the sensorimotor part of the internal globus pallidus (GPi) for Parkinson's disease and for dystonia (Vidailhet *et al.*, 2005). GPi stimulation and pallidotomy have shown to be effective to treat either generalized or focal primary dystonia. Its efficacy remains more controversial for secondary dystonias (Vidailhet *et al.*, 2005, 2009). Several studies have recently reported substantial benefits of the surgical treatment for severe TD. The results of lesioning surgery and deep brain stimulation in this indication are reviewed.

## II. Lesioning Surgery

A few studies have analyzed the efficacy of lesioning surgery in TD. All of them were open-labeled single case reports. The level of evidence for efficacy for such treatment is thus low. Lesioning surgery corresponded either to a pallidotomy or to a thalamotomy. In one case, a unilateral pallidotomy was performed and a 78% improvement of the Abnormal Involuntary Movement Scale (AIMS) score compared to the preoperative state is reported after a 2-month follow-up (Wang *et al.*, 1997). In another case, the improvement assessed by the Burke Fahn Marsden (BFM) scale was similar 8 months after a bilateral pallidotomy (Weetman *et al.*, 1997). One last case reports the results of a unilateral thalamotomy consisting in two lesions (one anterior and the other one posteromedial) (Hillier *et al.*, 1999). A 78% improvement of the BFM score was obtained after a 12-month follow-up. No side effects were reported in these three case reports. The data are summarized in Table I.

Table I
STUDIES HAVING ASSESSED THE EFFICACY OF LESIONING SURGERY ON TARDIVE DYSKINESIA[a].

| Series | Design | Number of Patients | Target | Follow-up | Scales | Preoperative Score (Mean) | Postoperative Score (Mean) | % of Improvement (Mean) | Reported side effects |
|---|---|---|---|---|---|---|---|---|---|
| Hillier *et al.* | Case report | 1 | Thalamus unilateral (1 anterior lesion + 1 posteromedial lesion) | 12 months | AIMS | 36 | 8 | 78 | None |
| Weetman *et al.* | Case report | 1 | GPi, bilateral | 8 months | BFM | 98 | 25 | 74 | None |
| Wang *et al.* | Case report | 1 | GPi, unilateral | 2 months | AIMS | 26 | 6 | 77 | None |

[a]*Abbreviations*: GPi: internal globus pallidus; BFM: Burke Fahn Marsden scale; AIMS: Abnormal Involuntary Movement Scale.

### III. Deep Brain Stimulation

The efficacy of deep brain stimulation in TD has been analyzed in a much greater number of series. Most of them are small and open-labeled series or single case reports but one controlled study has been carried out.

In all but one studies the ventroposterolateral part of the GPi has been the chosen target (Damier *et al.*, 2007; Eltahawy *et al.*, 2004; Franzini *et al.*, 2005; Kefalopoulou *et al.*, 2009; Kosel *et al.*, 2007; Sako *et al.*, 2008; Schrader *et al.*, 2003; Trottenberg *et al.*, 2005) (Fig. 1). In one series, the subthalamic nucleus has been the target for deep brain stimulation (Zhang *et al.*, 2006).

For bilateral GPi stimulation, the mean follow-up was between 5 and 21 months. The mean improvement, as assessed by the BFM or the AIMS scale was 63 + 17%. No permanent and disabling side effect was reported. The most frequently side effects observed were transitory and induced by the stimulation itself. In one case, GPi stimulation led to a bradykinesia concomitant to the reduction of abnormal movements (Schrader *et al.*, 2003); we have observed a similar effect in a few patients (Thobois, unpublished personal observation). Importantly no worsening of the underlying psychiatric disease was reported (Damier *et al.*, 2007; Eltahawy *et al.*, 2004; Franzini *et al.*, 2005; Kefalopoulou *et al.*, 2009; Kosel *et al.*, 2007; Sako *et al.*, 2008; Schrader *et al.*, 2003; Trottenberg *et al.*, 2005). One have to acknowledge that in all the cases the patients were carefully selected (i.e., with no more or stabilized psychiatric disorders) and closely monitored. In most of the patients, psychotropic drugs were reduced and the mood tended to improve (Damier *et al.*, 2007; Kosel *et al.*, 2007). The parameters of stimulation were variable ranging from low (40 Hz) to high (> 100 Hz) frequency, and a wide spectrum of pulse width and voltage. There is only one controlled study

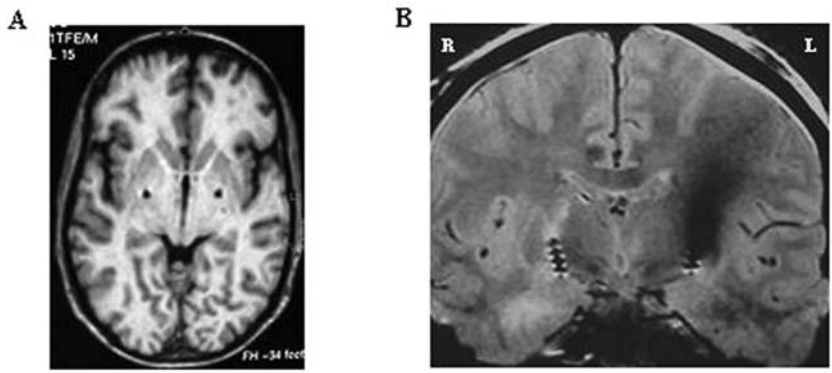


FIG. 1. Brain MRI, T1 sequences showing the final location of the electrodes within the GPi in a patient operated for tardive dyskinesia. A: axial view; B: coronal view.

Table II
Studies Having Assessed the Efficacy of Deep Brain Stimulation on Tardive Dyskinesia[a].

| Series | Design | Number of Patients | Target | Follow-up | Scales | Preop Score Mean | Postop Score Mean | % Improvement Mean | Stimulation Parameters Mean |
|---|---|---|---|---|---|---|---|---|---|
| Damier *et al.* | Double blind | 10 | GPi ventroposterolateral | 6 months | ESRS<br>AIMS | 73.1+10<br>25 + 3 | 28 + 4.5<br>11+2 | 61% ($p = 0.005$)<br>56% ($p = 0.006$) | 3.5 (0.2) V/150/ 130 Hz, monopolar |
| Eltahawy *et al.* | Case report Open labeled | 1 | GPi ventroposterolateral | 18 months | BFM | 52 | 21 | 60% | 2.6 V/210/40 Hz, monopolar |
| Franzini *et al.* | Case report Open labeled | 2 | GPi ventroposterolateral | 12 months | BFM | 53 | 6.5 | 87.7% | Unknown |
| Kefalopoulou *et al.* | Case report Double blind | 1 | GPi ventroposterolateral | 6 months | BFM<br>AIMS | 54<br>30 | 2.5<br>6 | 90.7%<br>76.7% | 2.5 to 3.6 V/250–450/185 Hz |
| Kosel *et al.* | Case report Open labeled | 1 | GPi ventroposterolateral | 18 months | BFM<br>BDI | 27<br>22 | 185<br>17 | 35% | 3.65 V/90/130 Hz |
| Sako *et al.* | Open labeled | 6 | GPi ventroposterolateral | 21 months | BFM | 40.2 | 5.7 | 85.8% | 2.9 (0.9) V/450/ 119 (28) Hz |
| Schrader | Case report Open labeled | 1 | GPi ventroposterolateral | 5 months | AIMS | 24 | 9 | 63% | 6.5 V/60/60 Hz |
| Trottenberg | Open labeled | 5 | GPi ventroposterolateral | 6 months | AIMS<br>BFM | <br>40 | <br>4 | 78%<br>90% | 2.7 (0.8)/111 (57)/ 144(22) Hz |
| Zhang | Case report Open labeled | 2 | STN DBS | 3 months | BFM | 26.5 | 2 | 90.6% | Unknown |
| Mean (SD) | | | | | | | | 63.3% + 17 | |

[a]*Abbreviations*: Gpi: internal globus pallidus; STN: subthalamic nucleus; BFM: Burke Fahn Marsden scale; AIMS: Abnormal Involuntary Movements Scale; ESRS: Extrapyramidal Symptoms Rating Scale.

having included 10 patients. Its results are in line with those obtained in open-labeled studies and clearly support the interest of bilateral GPi stimulation for treating severe TD (Damier *et al.*, 2007). Interestingly, this study demonstrated that GPi stimulation improved at the same time and to the same extent both dystonic and choreic-like abnormal movements observed in TD.

The efficacy of subthalamic nucleus stimulation was assessed in a 2-patient study. The beneficial effect was similar to the one obtained by GPi stimulation. Yet, no other study has confirmed this observation. The data are summarized in Table II.

The mechanism of action of GPi stimulation in TD remains poorly understood. Recent functional imaging studies have provided some interesting insights. Concomitantly to the improvement of the dystonia, GPi stimulation was shown to lead to a major reduction of the widespread brain overactivation (involving the prefrontal, premotor, motor and parietal cortices, and the cerebellum) associated with TD (Kefalopoulou *et al.*, 2009; Thobois *et al.*, 2008).

## IV. Conclusion

In severe and persistent TD, the surgical treatment, especially continuous bilateral GPi stimulation, can lead to a major improvement without any permanent disabling side effects. The level of improvement is comparable to the one obtained in DYT 1 primary dystonia (Vidailhet *et al.*, 2005). No psychiatric decompensation was reported in the studies but the patients suffering from TD were carefully selected, especially for not having any unstabilized psychiatric disorders, and were regularly monitored. It is a mandatory requisite for such a treatment. A close collaboration between neurologists, psychiatrists, and neurosurgeons is thus a key factor for selecting, operating, and following-up the patients appropriately and to obtain the level of efficacy observed in the reported studies.

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# HUNTINGTON'S DISEASE: CLINICAL PRESENTATION AND TREATMENT

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Huntington's disease (HD) is a devastating inherited neurodegenerative disease characterized primarily by progressive motor, cognitive, and psychiatric symptoms. It is caused by autosomal dominant inheritance of an expanded CAG repeat within the Huntington's gene on chromosome 4. In this chapter, we characterize the typical and variant motor phenotypes of the disease and then proceed to describe the cognitive and psychiatric profile. We then give an overview of a suggested multidisciplinary approach to the management of HD, emphasizing the fact that it is a disease which impacts on entire families rather than affecting individuals in isolation. We then describe the pharmacological and nonpharmacological options available for management of specific symptoms.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00013-4




0074-7742/10 $35.00

## I. Clinical Presentation and Genetics

### A. Introduction

Huntington's disease (HD) is a devastating inherited neurodegenerative disease characterized primarily by progressive motor, cognitive, and psychiatric symptoms. The mean age of onset of symptoms is 40 years, but juvenile (onset $< 20$ years) and older onset ($> 70$ years) forms also exist. The disease was originally named Huntington's chorea after George Huntington, who wrote the first detailed description in 1872. More recently, however, the name has changed to Huntington's disease to reflect the fact that chorea is not the only important manifestation of the disease: there are also many nonmotor symptoms which may in fact be more disabling and distressing than the motor symptoms. These are discussed in more detail below (Craufurd and Snowden, in press; Nance and Westphal, in press; Rothlind *et al.*, 1993). The pattern of symptoms can vary markedly between one patient and the next, even within the same family, so it is crucial to tailor management to every individual. In this chapter, we present a framework within which to address these issues.

### B. Epidemiology

HD occurs in all racial groups but is most common in people of Northern European origin. Its prevalence in the Western hemisphere is approximately 7-10/100,000 (Harper, 1992).

### C. The Genetics of Huntington's Disease

HD is a single-gene disease; it is caused by autosomal dominant inheritance of an expanded CAG trinucleotide repeat within the Huntingtin (*HTT*) gene on chromosome 4. This can be identified through genetic testing (The Huntington's Disease Collaborative Research Group, 1993). The *HTT* gene codes for the protein huntingtin, which is essential for normal neural development, though its functions are incompletely understood (Cattaneo *et al.*, 2005; Walker, 2007; Young, 2003). In HD, the expanded *HTT* gene codes for a mutant form of huntingtin protein which causes or contributes to the development of symptoms though various pathogenic mechanisms (Imarisio *et al.*, 2008).

A "normal" Huntington gene has fewer than 36 repeats. The gene is abnormal, or expanded, if it has 36 or more repeats, and CAG repeats of 40 or more will always cause HD. Genes with CAG repeat lengths of between 36 and 39 show reduced penetrance, which means that some people with these lengths will develop HD and some will not. Those who do develop disease are likely to develop later onset disease

(Langbehn *et al.*, 2004). An intermediate repeat length between 29 and 35 does not cause HD, but may expand into the pathogenic range in future generations.

The instability of intermediate alleles is one cause of apparently sporadic cases of HD, in which the disease develops in someone with no apparent family history of the disease. Apparently sporadic HD occurs in 6–8% of new cases of HD (Almqvist *et al.*, 2001; Siesling *et al.*, 2000), and can also be due to unexpected or unknown paternity, or a parent dying before they develop symptoms of the disease. Instability of the CAG repeat expansion can also cause "genetic anticipation," in which the CAG repeat length increases and causes the onset of the disease at a younger age in the affected offspring of someone with HD than in the parents themselves. Genetic anticipation is more common when the expanded allele is inherited from a father than from a mother. 90% of people with juvenile HD (with CAG repeats typically >60) inherit the mutation from their father (Harper and Jones, in press; Barbeau, 1970).

The following sections describe clinically relevant aspects of the genetics of HD. For more information, please see the authors' recent review of HD (Novak and Tabrizi, 2010).

1. *Genetic testing for Huntington's disease*

Genetic testing for HD is performed through quantification of the CAG repeat length in the *HTT* gene. We use the term "positive" genetic test result to refer to CAG lengths in the pathogenic fully penetrant expanded CAG repeat range of >39 repeats. Testing falls into two categories: *diagnostic testing* is carried out to confirm (or refute) the diagnosis in a patient with symptoms suggestive of HD, whereas *predictive testing* is carried out in a person who has no symptoms of HD, but who is at risk because of their family history. Predictive testing determines whether an individual carries the expanded *HTT* gene and will develop HD in the future. A positive predictive test result indicates that they will certainly develop HD at some point in the future (unless they die of another cause in the meantime), but does not tell them when this will happen or what the presenting symptoms will be. Predictive testing for HD is only performed in specialist genetic centers and follows internationally agreed guidelines (Craufurd and Harris, 1989; International Huntington Association, 1994; Went, 1990). These include an initial session of pretest counseling, followed by a period of reflection and then a second session of counseling. Posttest counseling must also be available and strict confidentiality must be observed. Both emotional and practical issues are discussed. Insurance and mortgage eligibility, for example, may be affected by a positive predictive test result.

2. *Reproductive options*

Deciding whether or not to have children is often difficult for people with or at risk of having an expanded *HTT* gene. A minority choose to have either prenatal

testing or preimplantation genetic diagnosis, which ensure that their child has a <1% chance of carrying the expanded *HTT* gene.

*Prenatal testing*

Prenatal testing is usually carried out via chorionic villus sampling between 11 and 13 weeks of pregnancy. Pretest counseling is important: potential parents need to be sure that they will terminate the pregnancy if their fetus is found to have an expanded *HTT* gene, otherwise their child will grow up in the shadow of a predictive test for which they did not consent. This would violate the autonomy of the child.

*Preimplantation genetic diagnosis (PGD)*

Preimplantation genetic diagnosis (PGD) is available through specialist units. In PGD, embryos are created using normal IVF procedures and then tested for the expanded *HTT* gene. Unaffected embryos are implanted. Overall, about one in five cycles results in a live birth, but success rates vary.

*Exclusion (nondisclosing) prenatal testing and PGD*

Exclusion (nondisclosing) prenatal testing or PGD can be carried out for couples in which one partner is at risk but does not wish to have a predictive test themself: the potential parents do not find out their own HD gene status. Linkage techniques are used. A "high risk" result means that the fetus is at 50% risk of developing HD——the same as its at-risk parent. The couple undergoing this test therefore chooses to terminate a pregnancy at 50% risk. Clearly this test requires detailed discussion with the couple beforehand.

## D. The Course of the Disease and its Relationship with CAG Repeat Length

An individual with an expanded HTT gene will usually develop symptomatic, or "manifest," HD in their 30s or 40s. This will generally progress to end-stage disease over 15–30 years. The onset of manifest HD is currently defined as the point at which characteristic motor signs develop (Huntington Study Group, 1996); prior to this point, the individual is classed as a "premanifest gene carrier." The distinction between premanifest and manifest disease is somewhat arbitrary, however, as most patients develop some or all of cognitive, psychiatric or subtle motor symptoms during the premanifest ("prodromal") period, and this often occurs many years before any "hard" motor signs are seen (Paulsen *et al.*, 2006, 2008; Tabrizi *et al.*, 2009). Patients may present initially with any symptom. Chorea and loss of balance are early symptoms that patients themselves often

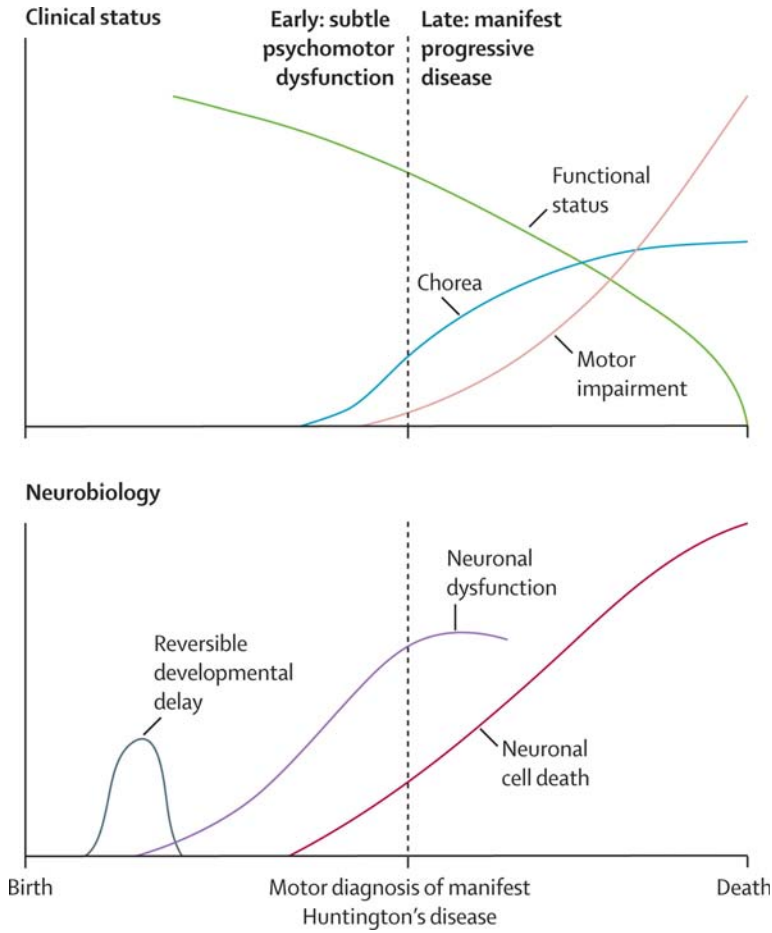


FIG. 1. Progression of Huntington's disease over a patient's lifespan (reproduced from "Huntington's disease: from molecular pathogenesis to clinical treatment" by CA Ross and SJ Tabrizi by kind permission of the publishers (Ross and Tabrizi, 2011)).

notice, though frequently families notice cognitive or personality changes prior to this.

The typical pattern of disease progression is shown in Fig. 1 (Ross and Tabrizi, 2011).

The expanded CAG repeat length is related to age of onset of disease at a population level: the longer the CAG repeat length, the earlier the onset of symptoms tends to be (Andrew *et al.*, 1993; Brinkman *et al.*, 1997; Duyao *et al.*, 1993; Snell *et al.*, 1993; Stine *et al.*, 1993; Langbehn *et al.*, 2004). However, repeat

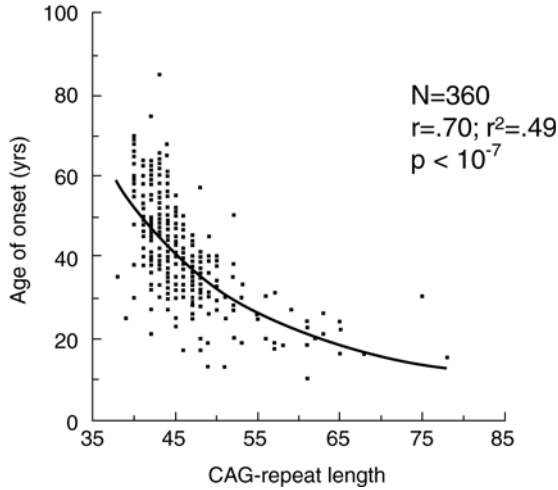


FIG. 2. The correlation between CAG repeat length and age of symptom onset (reproduced from "The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease" by SE Andrew *et al.* by kind permission of the publishers (Andrew *et al.*, 1993)).

length only accounts for between 50 and 70% of this variance, so disease onset in an individual cannot be predicted reliably through genetic testing (Wexler *et al.*, 2004). This can be seen in Fig. 2 (Andrew *et al.*, 1993). Use of the CAG repeat length to predict disease onset is useful in Huntington's disease research, but has minimal relevance to the management of individual patients. The CAG repeat length is also associated with the rate of disease progression, but less strongly than with the age of symptom onset (Rosenblatt *et al.*, 2006; Ravina *et al.*, 2008).

## E. Staging of Huntington's Disease

In clinical practice, staging in HD is usually based on the HD-specific Total Functional Capacity (TFC) scale (Shoulson and Fahn, 1979). Staging is based on functional ability in recognition of the fact that some symptoms of HD are more functionally disabling than others (Rothlind *et al.*, 1993). A score is given according to an individual's ability to function independently in each of four domains—occupation, finances, domestic chores and activities of daily living—and the level of nursing care they need. The scale covers all stages of the disease, ranging from minimal functional impairment to requiring complete support in every domain.

The Unified Huntington's Disease Rating Scale (UHDRS) (Huntington Study Group, 1996) is a clinical rating scale used primarily for research purposes. It is used to assess patient ability in four domains: functional capacity (assessed using the Shoulson–Fahn TFC scale), motor function, cognition, and behavior.

## F. Huntington's Disease is a Disease of the Family

HD has a profound impact on those around an affected individual as well as on that individual him or herself. The inexorable progression of the disease is painful for families and friends to watch, and most affected families will include more than one affected individual, with several more at risk of developing the disease in the future. In addition, the burden of being first a carer and later a patient is handed down through the generations; for many people, being tested positive for the HD gene carries the dual consequences of being diagnosed with a disease that has taken the life of a parent, and passing on the burden of being a carer and perpetuating the cycle to one's own children. HD is often little understood by those who have not experienced it first hand, so having supportive family members who are familiar with the disease can be particularly important.

## G. Overall Principles of Management of Huntington's Disease

As mentioned earlier, HD can cause markedly different patterns of symptoms from one patient to the next, even within the same family. It is therefore crucial to tailor management to each individual, and to ensure that the priorities of the patient are listened to when formulating management plans. Patients may be less concerned by their chorea than their family is, for example (and, in fact, it is very common for people with mild chorea not to be aware of it at all). If this is the case, it is important not to medicalize the person with HD or to expose them to potential drug side-effects by attempting to treat a symptom which is not actually causing a problem: the aim of current treatments for HD is to manage symptoms and improve quality of life (Novak and Tabrizi, 2010). One caveat to this, however, is that it is common for patients to lack insight into their symptoms; carer input is therefore invaluable when assessing someone with HD and deciding which symptoms are causing difficulties. The social and financial impact of HD can also be considerable and this should always be considered when assessing anyone with HD.

There are no treatments available to slow disease progression yet, but there are many effective options for symptomatic management, including both

pharmacological and nonpharmacological measures (Mestre *et al.*, 2009; Phillips *et al.*, 2008). The evidence base for drugs in HD is very small (Adam and Jankovic, 2008; Bonelli and Hofmann, 2007; Mason and Barker, 2009; Priller *et al.*, 2008) so the choice of pharmacological agents is based mainly on clinical experience. Nonpharmacological measures are often more helpful than pharmacological measures (Nance, 2007; Nance and Westphal, in press); once again, limited evidence is available, but recommendations are based on extensive clinical experience. Further details are given in subsequent sections of this chapter.

The provision of care in HD usually requires a multidisciplinary approach (Nance, 2007; Nance and Westphal, in press). Patients usually benefit from referral to a specialist multidisciplinary HD clinic where possible, from which they can access care from a range of healthcare professionals experienced in the management of HD. Support from professionals in the community remains vital, and optimal care is typically provided from a multidisciplinary team which includes some or all of the following: general practitioners, neurologists, geneticists, psychiatrists, physiotherapists, occupational therapists, speech and language therapists, dieticians, community mental health teams, and social workers (Novak and Tabrizi, 2010). Table I gives an overview of the multidisciplinary nonpharmacological management of HD. Early involvement is recommended so that patients learn how to manage their symptoms while they still have the cognitive capacity to adapt and learn new skills.

Table I

Multidisciplinary Nonpharmacological Management of Huntington's Disease (reproduced from "Huntington's disease" by MJU Novak and SJ Tabrizi by kind permission of the publisher).

| Feature of Disease | Examples of Management Measures |
|---|---|
| Gait disturbance and chorea | Physiotherapy to optimize and strengthen gait and balance, and to assess for walking aids; occupational therapy assessment to modify home environment to improve safety; weighted wrist bands to combat limb chorea |
| Cognitive symptoms | Ensure every day has a structure to overcome apathy and difficulty in initiating activities (occupational therapy can advise on this); maintain routines to reduce need for flexibility |
| Social support | Carers to help at home; residential or nursing home care; day centers to maintain social interactions |
| Communication | Speech and language therapy to optimize speech, and later in disease to assess for communication aids; ensure patient has time to comprehend and respond to speech, and that information is presented simply |
| Nutrition | Speech and language therapy to advise on safest food consistencies at different stages of disease, and, in later disease, to advise on need to consider enteral nutrition; dietician to optimize nutritional intake, especially adequate calorie intake; minimize distractions to optimize swallowing safety |
| Psychological therapy | Develop strategies to deal with cognitive and/or emotional challenges of disease using counseling or CBT |

Advisors and local groups linked with HD support organizations can play an invaluable role in supporting patients and their families. These organizations include the HD Association (HDA) in England and Wales and the HD Society of America (HDSA); many other countries have similar organizations (the International Huntington Association website, www.huntington-assoc.com, includes contact details for these). Care advisors can also provide educational input for healthcare professionals caring for people with HD. In some countries, specific groups exist to support young people affected by HD and can be accessed via the main support organizations.

## II. The Clinical Phenotype and its Management

### A. The Motor Disorder

The motor symptoms of HD can be divided into two categories: added involuntary movements such as chorea, and impaired voluntary movements, which cause limb incoordination and impaired hand function. These symptoms are worsened by loss of postural reflexes. The pattern of symptoms tends to change over time in typical adult-onset HD: typically chorea dominates in early disease, but then tends to decline as the disease progresses, with dystonia, rigidity, and bradykinesia then becoming more marked (Novak and Tabrizi, 2010). In general, these later symptoms tend to be more functionally incapacitating than the more readily recognized chorea. The change in symptomatology over time is particularly important when considering pharmacotherapy. Movement-suppressing medications used in the earlier stages of the disease may exacerbate the impaired movements that develop later on. They will often need to be reduced, and eventually stopped, so regular reassessment is vital.

A summary of the pharmacological treatments of the movement disorder is given in Table II.

#### 1. *Chorea*

The first step in managing the movement disorder is to decide whether symptoms need treating. This is particularly relevant for chorea; patients are often not bothered by early chorea, and may not even be aware of it (Novak and Tabrizi, 2010). As chorea develops, however, it tends to become more problematic and to interfere with voluntary activities like writing or eating, and frequently causes falls. Chorea can also be distressing in itself, and patients often find themselves accused of drunkenness by people unaware of their diagnosis. These issues may be indications for treatment.

Table II
Symptomatic Management of the Huntington's Disease Motor Disorder (reproduced from "Huntington's disease" by MJU Novak and SJ Tabrizi by kind permission of the publisher).

| Symptom | Drug Class | Medication | Main Adverse Effects and Treatment Notes |
|---|---|---|---|
| Chorea | Atypical neuroleptics | Olanzapine | Sedation, parkinsonism, tardive dyskinesias but less risk than with older neuroleptics raised triglycerides, weight gain from increased appetite which may be beneficial in HD. Caution should be exercised in patients with diabetes and blood glucose monitored. May rarely cause prolonged QT interval. Useful if also significant agitation, irritability and anxiety |
| | Atypical neuroleptics | Risperidone | As above but less effect on increasing appetite. |
| | Atypical neuroleptics | Quetiapine | As above, less effects on lipids and glucose |
| | Older neuroleptics | Sulpiride | Agitation, dystonia, akathisia, sedation, hypotension, dry mouth, constipation. |
| | | Haloperidol | Sedation, more parkinsonism, dystonia, akathisia, hypotension, constipation, dry mouth, weight gain, higher risk of neuroleptic malignant syndrome than atypical neuroleptics |
| | Dopamine depleting agents | Tetrabenazine | Depression and sedation |
| Myoclonus<br>Chorea<br>Dystonia<br>Rigidity<br>Spasticity | Benzodiazepines | Clonazepam | Sedation, ataxia, apathy, cognitive impairment may be exacerbated, withdrawal seizures |
| Myoclonus | Anticonvulsant | Sodium valproate | GI disturbance, weight gain, blood dyscrasia, hyperammonemia |
| | | Levetiracetam | GI disturbance, rash, mood changes, myalgia |
| Rigidity (particularly associated with juvenile HD or young adult-onset Parkinsonian phenotype) | Amino acid precursor of dopamine | Levodopa | GI disturbance, postural hypotension, insomnia, agitation, psychiatric symptoms |
| Rigidity<br>Spasticity | Skeletal muscle relaxants | Baclofen<br>Tizanidine | Sedation, drowsiness, confusion, GI disturbances, hypotension |
| Bruxism<br>Dystonia | Inhibits acetylcholine release at neuromuscular junction to cause muscle paralysis | Botulinum toxin | May paralyze nearby muscles |

Nonpharmacological interventions should be considered first. Chorea often varies with posture or positioning, and assistive devices, such as padded chairs or wrist and ankle weights to reduce chorea amplitude, may be helpful. Wearing shoes with nonslip soles and installing grab rails around the home can improve safety, and occupational therapy assessment to assess the home environment is often extremely useful. Physiotherapy is also helpful to optimize mobility and can be beneficial at an early point in the disease to preserve mobility and independence for as long as possible (Novak and Tabrizi, 2010).

Stress, anxiety, and depression can all worsen chorea, so measures to treat these are often helpful. Creating a calm and predictable environment is also beneficial.

If these measures are not sufficient to control symptoms, medications can be tried. These are unlikely to obliterate chorea, but can dampen down symptoms considerably. There are three different classes of medication that are commonly used: neuroleptics (e.g., olanzapine, risperidone), benzodiazepines (e.g., clonazepam, diazepam), and dopamine depleting agents (e.g., tetrabenazine). As mentioned above, there have been very few systematic clinical trials comparing the efficacy of these drugs and so management is primarily based on the opinion of experts rather than on a substantial evidence base. Choice is often dependent on consideration of the side-effect profile of each drug class.

Tetrabenazine has the best evidence of efficacy in HD and has been shown to reduce chorea in a randomized controlled clinical trial (Huntington Study Group, 2006). It is frequently the first choice of medication for uncomplicated chorea as although it shares some of the side effects of the neuroleptics, they tend to be milder. In addition, tetrabenazine has not been shown to cause tardive dyskinesia. (Tardive dyskinesia is of particular concern in HD because it can be difficult to detect in someone with a movement disorder.) Tetrabenazine can, however, exacerbate or trigger psychiatric symptoms so should be avoided in patients with a history of depression or other psychiatric disorders; in these patients, or in those in whom symptoms are not controlled with tetrabenazine, neuroleptics are helpful.

CYP2D6 (cytochrome P450 2D6) is integral in the metabolism of tetrabenazine. To avoid reduced clearance, tetrabenazine should therefore be used with care in patients who are also taking drugs with a CYP2D6 inhibitory effect. CYP2D6 inhibitors include fluoxetine, paroxetine and fluvoxamine; noninhibitory alternatives include citalopram, escitalopram and sertraline (Guay, 2010).

Neuroleptics are used to treat chorea by harnessing the movement suppression that is seen as an undesirable side-effect when the same drugs are used to treat psychosis. In the past, "typical" neuroleptics like haloperidol were frequently used, but the "atypical" neuroleptics are now more commonly used. These include olanzapine risperidone and quetiapine. They are usually better tolerated with fewer extrapyramidal side-effects (such as unacceptable levels of rigidity and dystonia) and a lower incidence of tardive dyskinesia than the older neuroleptics.

The atypical neuroleptics also promote weight gain, suppress irritability, and mood swings, and improve sleep—all of which are useful side-effects in HD. They should be started at the lowest dose, and can be gradually titrated up as needed. Olanzapine and risperidone can both cause hypercholesterolaemia and hyperlipidaemia and are associated with an increased risk of stroke in elderly patients with dementia (Ballard and Howard, 2006); it is therefore important to consider a history of stroke or transient ischemic attack in older patients before using them and to monitor glucose and lipid levels during treatment.

The typical neuroleptics are also still used to suppress chorea. Sulpiride, for example, is a good suppressor of chorea, but can cause agitation and Parkinsonism. Haloperidol causes even more frequent Parkinsonian side effects, and also causes tardive dyskinesia. All neuroleptics carry a risk of neuroleptic malignant syndrome, but this is greater with the typical than the atypical neuroleptics. (Neuroleptic malignant syndrome is a rare, but life-threatening reaction characterized by acute onset of delirium, rigidity, and fever, often accompanied by leukocytosis and elevated CPK. Families should know about this so that patients can be given prompt medication attention if this develops.)

Benzodiazepines can also be used for treatment of chorea, but they cause sedation and can have depressant effects on cognition. For these reasons, they are better avoided in the longer term. Amantadine can also be used for intractable chorea. Its main side-effects are ankle edema, worsening of confusion, and livedo reticularis.

2. *Dystonia, spasticity, rigidity, and bradykinesia*

As mentioned earlier, rigidity, spasticity and bradykinesia tend to emerge later in adult-onset HD, often becoming more prominent as chorea declines. In juvenile HD, however, a more Parkinsonian phenotype is often present from the onset of the disease. These symptoms usually cause marked functional impairment and often impair gait, leading to falls and the need for a wheelchair.

Dystonia may be a symptom of HD, or a side effect of a neuroleptic. Different presentations include twisting, tilting, or turning of the neck (torticollis), involuntary arching of the back (opisthotonos), and arching of the feet.

A variety of medications have been used to treat rigidity, spasticity, and dystonia. As with chorea, these do not obliterate symptoms, but may partially suppress them. Benzodiazepines or baclofen may relieve stiffness, but may also increase bradykinesia. Anti-Parkinsonian medicines such as levodopa-containing compounds or amantadine can also be helpful. Tizanidine sometimes improves spasticity. All of these medicines may cause delirium, however, and even if initially helpful, may lose their efficacy after several months; improving mobility with the help of a physiotherapist and preventing contractures is frequently the most important aspect of management. Botulinum toxin injections are not often used

but may be useful if severe rigidity of a particular small muscle or group of muscles is impairing function.

3. *Myoclonus and tics*

Myoclonus is sudden brief jerking of groups of muscles. It is most common in juvenile-onset HD, and may be mistaken for a seizure. It may not be especially disabling or distressing, but if needed, can be dampened down with clonazepam, sodium valporate, or levetiracetam. Tics are brief, intermittent stereotyped movements such as blinking, nose twitching, head jerking, or transient abnormal postures. Tics can also cause sounds like sniffs, snorts, grunts, coughs, and sucking through involvement of respiratory and vocal structures. As with chorea, patients often do not notice their tics although other people who spend a lot of time with them can find the constant noise of a persistent vocal tic irritating; it is important to explain that, just like chorea, tics are not something that patients can control. Tics do not usually need treatment, but neuroleptics, benzodiazepines, or SSRIs may help to suppress them.

4. *Akathisia*

Akathisia is an extremely uncomfortable internal sense of restlessness, which may cause patients to pace or to be unable to sit still. It can be caused by neuroleptics and can look like agitation or anxiety; this means that a vicious circle can be created if the causative neuroleptic is increased to treat perceived agitation or anxiety.

## B. The Cognitive Disorder

Cognitive symptoms tend to begin insidiously, but can have a profound effect even at an early stage. In 1993, Rothlind *et al.* assessed the effect of cognitive and motor symptoms on the ability of 67 individuals with early HD to carry out activities of daily living and found that cognitive impairment was associated with reduced functional ability independent of motor impairment (Rothlind *et al.*, 1993). Additionally, many patients and their families can identify problems at work and in personal relationships that can, with hindsight, be attributed to development of the cognitive syndrome many years before the onset of motor symptoms. Patients themselves may not be aware of these symptoms (or may be in denial about them), and this lack of insight may confound the problem.

The first step in managing cognitive symptoms is therefore to identify areas of difficulty and to recognize that they might be due to HD. This can be difficult to address in at-risk individuals who do not wish to know their gene status and needs

to be introduced gently. There are no pharmacological treatments for cognitive symptoms, but coping strategies can often be adopted to overcome or compensate for problems. Encouraging patients to continue to exercise their mind, for example by doing crosswords or other puzzles, is advised across the disease span, and cognitive behavioral therapy (CBT) can be very helpful for people with early cognitive or behavioral symptoms who have insight and are motivated to engage with the therapy. This can also be a useful way for premanifest individuals to learn cognitive strategies that will stand them in good stead once cognitive and psychiatric symptoms become manifest. (We are aware of no controlled trials of CBT in HD, though one case study has reported benefit for a premanifest individual who underwent CBT to manage depression and anxiety after a positive predictive gene test (Silver, 2003)).

The following sections summarize some of the features of the cognitive syndrome.

1. *Executive dysfunction*

Executive functions are the high-level cognitive processes that control other aspects of cognitive function. These almost invariably decline in HD. Typically, patients report difficulty with multi-tasking and concentration. Thinking-style becomes more concrete and less efficient, and planning, initiation and organization of time, thoughts and activities become harder. External structure can be provided through establishing regular routines and keeping diaries and "to do" lists; this helps patients to organize activities.

Difficulty with multitasking is a common early sign of HD, and can result in significant problems in day-to-day function. Many workplaces require rapid switching of attention, for example; HD-related deficits can result in patients making mistakes in this type of environment. Concentrating in a busy office becomes harder and tension develops in relationships with colleagues. This, in turn, increases stress and exacerbates the underlying problem with concentration. Once these issues have been identified, however, appropriate compensatory strategies can be devised. Moving into a quiet office and reducing workload, for example, may enable someone to stay in work and preserve their independence for as long as possible. Employers have a statutory duty to optimize the working environment for people with a disability where possible, though this needs to be approached sensitively and support and advice offered (Novak and Tabrizi, 2010).

2. *Psychomotor symptoms*

People with HD are frequently impulsive and develop psychomotor perseveration.

3. *Visuospatial and perceptual deficits*

HD causes subtle visuospatial perceptual changes (Craufurd and Snowden, in press; Paulsen *et al.*, 2008). In particular, patients' perception of their own bodies in relation to the rest of the world can be impaired; this may contribute to trips, falls and bumping into furniture.

4. *Learning and memory*

Memory loss and difficulty in learning new skills are common. Strategies to deal with this include providing cues to jog the memory, and again, keeping "to do" lists and diaries and maintaining regular routines.

5. *Dementia in Huntington's Disease*

As cognitive dysfunction progresses, patients can develop severely limiting frontal and subcortical dementia. It is important to remember, however, that some people with profound motor symptoms will remain able to comprehend and evaluate information and make decisions for themselves, even if they find this difficult to communicate. Assessment by a clinical psychologist can be extremely helpful in order to explore this more fully and to evaluate the extent of an individual's capacity.

## C. The Psychiatric Disorder

Psychiatric symptoms are common in HD and, like cognitive symptoms, often precede motor onset by many years. They frequently lead to considerable distress and difficulty for patients and their families/carers, who typically find them more difficult to deal with than the physical symptoms. It is important to recognize psychiatric symptoms so that symptomatic treatment can be offered, and to acknowledge that symptoms are probably caused by HD. Detection of psychiatric symptoms may be difficult later in the disease as diagnoses may be obscured by other symptoms; depression, for example, may be difficult to detect in a patient who has altered facial expressions and tone of voice. Conversely, metabolic symptoms such as weight loss and sleep disturbance may be misattributed to depression (Novak and Tabrizi, 2010).

HD can cause a wide range of psychiatric symptoms. A survey of 52 patients with HD was found to have symptoms with the frequencies shown in Table III. (Paulsen *et al.*, 2001).

A summary of the pharmacological treatment of psychiatric symptoms in HD is given in Table IV.

Table III
Frequency of Neuropsychiatric Symptoms in Huntington's Disease as Measured by the Neuropsychiatric Inventory (NPI; Cummings *et al.*, 1994) (adapted from "Neuropsychiatric aspects of Huntington's disease" by JS Paulsen *et al.* by kind permission of the publisher).

| Symptom | Frequency | Mean | SD |
|---|---|---|---|
| Dysphoria | 69.2 | 3.12 | 3.46 |
| Agitation | 67.3 | 2.88 | 3.32 |
| Irritability | 65.4 | 2.63 | 3.11 |
| Apathy | 55.8 | 2.79 | 4.02 |
| Anxiety | 51.9 | 1.96 | 3.14 |
| Disinhibition | 34.6 | 1.29 | 2.77 |
| Euphoria | 30.8 | 1.04 | 2.27 |
| Delusions | 11.5 | 0.75 | 2.63 |
| Aberrant motor | 9.6 | 0.60 | 2.18 |
| Hallucinations | 1.9 | 0.23 | 1.66 |

1. *Depression*

Depression is extremely common in HD and occurs as an intrinsic feature of the disease rather than merely as a response to being diagnosed with an incurable disease. A recent survey of 2,835 patients with HD found that 40% currently had symptoms of depression and 50% reported having sought treatment for depression in the past (Paulsen *et al.*, 2005). 10% of those surveyed had made at least one suicide attempt in the past.

Treatment is with standard antidepressant medications. While there is not an established evidence base for the treatment of depression in HD, our experience is that antidepressants are frequently very effective (Novak and Tabrizi, 2010). An SSRI such as citalopram is generally used as first line treatment, though stimulating SSRIs such as fluoxetine should be avoided as they can cause hyperstimulation and exacerbate anxiety, both of which are common in HD. If insomnia is a problem, a sedating antidepressant at night instead (e.g. mirtazapine) can be useful. Psychological therapies, such as CBT, can also be helpful in well-selected patients, and support from local community mental health teams is often invaluable.

2. *Suicide risk*

Patients with HD are more likely than the general population to commit suicide according to a meta-analysis of studies that reported on mortality associated with mental disorders (standardized mortality ratio of 290) (Harris and

Table IV
Symptomatic Management of Psychiatric Symptoms in Huntington's Disease (reproduced from "Huntington's disease" by MJU Novak and SJ Tabrizi by kind permission of the publisher).

| Symptom | Drug Class | Medication | Main Adverse Effects and Treatment Notes |
|---|---|---|---|
| Psychosis | Atypical neuroleptics | Olanzapine, Risperidone, Quetiapine | See above. Careful use in the elderly where there is increased risk of stroke with olanzapine and risperidone |
| Treatment-resistant psychosis | Neuroleptics | Clozapine | As for the other neuroleptics, plus agranulocytosis, myocarditis and cardiomyopathy. Requires blood monitoring |
| Psychosis with prominent negative symptoms | Neuroleptics | Aripiprazole | Parkinsonism, akathisia, drowsiness, GI disturbance, tremor, blurred vision |
| Depression, anxiety, OCB, irritability, aggression | Selective serotonin reuptake inhibitors (SSRI) | Citalopram | GI disturbance, hypersensitivity reactions, drowsiness, syndrome of inappropriate antidiuretic hormone secretion (SIADH), postural hypotension |
| | | Fluoxetine | As for citalopram, sleep disturbances |
| | | Paroxetine | As for other SSRIs, raised cholesterol |
| | | Sertraline | As for other SSRIs |
| | Presynaptic $\alpha_2$-antagonist, increases central noradrenaline and serotonin activity | Mirtazapine | Weight gain, edema, sedation, headache, dizziness, tremor. Useful when insomnia is a problem as it is sedating. |
| | Serotonin and noradrenaline reuptake inhibitor | Venlafaxine | Hypertension, GI disturbance, hypersensitivity reactions, drowsiness, agitation, SIADH, palpitations |
| Irritability, aggression | Neuroleptics | Olanzapine, Risperidone, Quetiapine | See above |
| Altered sleep-wake cycle | Hypnotics | Zopiclone Zolpidem | Drowsiness, confusion, memory disturbance, GI disturbance |
| Mood stabilizers | Anticonvulsants | Sodium valproate | See above |
| | | Lamotrigine | Hypersensitivity reactions, blood dyscrasias, dizziness, GI disturbance, depression |
| | | Carbamazepine | Hypersensitivity reactions, drowsiness, blood dyscrasias, hepatitis, hyponatremia, dizziness, GI disturbance |

Barraclough, 1997). A survey of 4,171 carriers of the Huntington's gene with premanifest and manifest disease found that 17.5% had suicidal thoughts at or around the time of assessment and 10% of those surveyed had made at least one suicide attempt in the past (Paulsen *et al.*, 2005). It is therefore vital to ask depressed patients whether they have been experiencing suicidal thoughts. Suicidal ideation was highest among (a) gene carriers who were nearing the threshold of being diagnosed with manifest disease (i.e. those with soft motor signs of HD) and (b) among those who were beginning to lose their functional ability and independence (i.e. those with stage 2 disease). Risk factors for suicide in HD include depression and impulsivity (Craufurd and Snowden, in press). Some people with HD also have suicidal thoughts in the absence of depression (Lipe *et al.*, 1993): for some, thoughts of suicide appear to be a rational response to their imminent loss of independence (Novak and Tabrizi, 2010).

3. *Anxiety*

Anxiety is also common in HD but can respond to treatment with nonstimulating SSRIs, buspirone or benzodiazepines.

4. *Irritability and agitation*

As seen in Table III, these are also common symptoms of HD. Both respond well to neuroleptics - olanzapine, for example, is very effective and, as described earlier, has beneficial side-effects. Behavioral strategies are also invaluable and carers should be encouraged to create a calm and structured environment and to avoid confrontation wherever possible. It is also helpful to avoid situations that trigger outbursts, but if this is not possible, short-term use of benzodiazepines (e.g. a low dose of clonazepam) can be useful to reduce agitation and anxiety. These strategies can be difficult for carers to maintain, and support groups like local Huntington's Disease Association (HDA) meetings can be valuable sources of support and suggestions.

5. *Apathy*

Apathy is also a challenging symptom to manage. It can be difficult to differentiate from depression and a trial of antidepressants may be worth considering if there is uncertainty about this. It is helpful to gently impose structure on the day as patients often find that having an appointment to aim for, such as coffee with a friend, is a helpful way to initiate and organize their behavior (Novak and Tabrizi, 2010). Patients suffering from apathy can find it particularly difficult to initiate activities, but are often able to participate fully with encouragement and support once they get started on things.

6. *Obsessive-compulsive behaviors and perseveration*

Obsessive-compulsive thoughts and behaviors are also relatively common in HD (Cummings and Cunningham, 1992; Paulsen *et al.*, 2001; Rosenblatt and Leroi, 2000). These take three main forms: obsessions related to other people (e.g. suspicions of infidelity), obsessions which are related to the self (e.g. fixations on bowel or bladder function), and ritualistic behaviors (e.g. preoccupations with specific routines) (Tabrizi *et al.*, 2011). It is generally not helpful to be confrontational; gentle redirection can however be helpful. Neuroleptics such as olanzapine can also improve symptoms, as can antidepressants. The choice of drug class should be based on the pattern of concurrent symptoms: neuroleptics would be a good choice in a patient who also has agitation, for example. In patients with little or no cognitive impairment, CBT can also be useful.

7. *Sexuality*

Sexuality often remains unchanged in HD, though it is also common for sex drive to decline. A minority of people with HD, almost invariably men, develop hypersexuality. An open and supportive atmosphere is important to explore strategies to deal with this. In general, a behavioral approach is used to broach issues arising from altered sexuality, but neuroleptics such as olanzapine can help to reduce hypersexuality if needed. It is also important to remember that many patients with HD are on medications such as SSRIs which can cause sexual dysfunction; these should be reviewed if sexual dysfunction is a problem.

8. *Psychosis*

Psychotic symptoms are rare in HD, but delusions and hallucinations can occur (Paulsen *et al.*, 2001; Rosenblatt and Leroi, 2000). Any precipitating factors should be assessed and treated if present, and neuroleptic treatment instigated as necessary. The choice of specific neuroleptic depends on the concurrent symptom profile (Rosenblatt and Leroi, 2000).

## D. Communication

Communication can be impaired by both dysarthria and cognitive dysfunction. Dysarthria is mainly caused by incoordination of voluntary oromotor muscle movement and, as with most other HD symptoms, is often worse when an affected individual is tired or under stress. Cognitive symptoms which contribute to communication problems include word-finding difficulties and an inability to initiate or structure speech.

Maintaining communication is vital, and there are a number of strategies which can help to optimize communication. When giving information to someone with HD, it is important to be clear. Decision-making can be challenging, so present an individual with simple and clear choices rather than asking open-ended questions ("would you like a sandwich or a biscuit?" rather than "what would you like to eat?"). Use uncomplicated language and allow the patient plenty of time to reply. Prompting can be helpful to overcome difficulties in initiating speech. People with HD are often distracted by extraneous information in their environment, so if communication is difficult, it can help to move to a quieter space where distractions are minimized. If the listener cannot understand the person with HD, asking them to repeat things or phrasing questions differently may help. As communication problems progress, speech aids such as communication boards can be helpful. People with HD frequently often understand far more than initial attempts at communication suggest, however; it is important to keep this in mind and to look actively for ways to optimize communication.

Speech and language therapy (SALT) is frequently helpful and can be very useful to maximize speech clarity in the early stages of the disease and to teach patients and their families communication strategies while they have the cognitive ability to learn them.

### E. Swallowing Problems

Swallowing problems also arise from both motor and cognitive dysfunction. Oromotor incoordination and distractibility are common factors. Food often goes down the wrong way when patients are distracted while eating; minimizing distractions such as talking or watching television during mealtimes is helpful and patients should be advised to eat slowly, sitting upright and concentrating on chewing and swallowing. Meals may need to be supervised with carers reinforcing this advice, and it is important to make sure that any dentures fit well. As the disease progresses, people with HD usually need to modify their diets to avoid troublesome foods, and food consistencies can be altered to reduce the chance of aspiration, for example by adding thickener to fluids. SALT referral is important at an early stage for advice on how to reduce the risk of aspiration and it is useful for carers to be trained in how to perform airway clearing maneuvers in case choking occurs.

Eventually patients may become unable to swallow anything safely and PEG insertion may be considered. This is often a highly emotive subject and the issue should be raised with patients and their carers while patients are still able to eat to avoid crisis point being reached. PEG insertion does reduce the risk of aspiration but patients may choose not to have this. It may be the subject that triggers a patient to make an advanced directive; a significant number of individuals decide against PEG insertion and planning in advance with an advanced directive can be invaluable in

avoiding the need to discuss this when disease has advanced to the point when patients are unable to communicate and a decision is needed urgently. This is discussed in more detail in the section on advanced disease and end of life issues.

### F. Nutrition

Widespread metabolic and endocrine changes are increasingly recognized in HD, and their management can be difficult. The disease creates a catabolic state, resulting in weight loss being a prominent feature of HD. This may begin in the prodromal period of the disease. As the disease progresses, massive weight loss may occur unless calorie intake is increased. People with HD often need a vastly increased daily calorie intake—up to 4000 calories a day—to maintain a stable weight. Anecdotally, weight loss has a negative impact on other symptoms, so avoiding it and monitoring weight carefully is a priority.

Diet can be supplemented with high calorie foods such as cream and chocolate in the first instance. Nutritional supplements are useful as the disease progresses, and referral to a dietician to optimize calorie intake while maintaining as well balanced and enjoyable a diet as possible can be helpful. As people with HD are encouraged to eat a high calorie/high sugar diet, close attention should be paid to oral hygiene and regular dental reviews encouraged.

### G. Sleep

Sleep disturbance is common in HD (Arnulf *et al.*, 2008; Hansotia *et al.*, 1985; Videnovic *et al.*, 2009; Wiegand *et al.*, 1991). Low mood, anxiety, and added movements are all secondary causes of insomnia, and in addition, primary sleep disturbance is a significant feature of the disease itself. The sleep-wake cycle becomes disordered and daytime somnolence is common.

Sleep hygiene measures are the first step in managing this; avoiding afternoon napping and keeping regular hours for going to bed and getting up are particularly important things to mention to patients, especially as the cognitive changes of HD mean that they can find it difficult to impose structure on daily routines. Treating chorea and mood disturbances as required can improve sleep, and a small dose of olanzapine or clonazepam at night as sedation may also be helpful.

### H. Metabolic and Endocrine Features

As mentioned earlier, widespread metabolic and endocrine changes are increasingly recognized in HD. Weight loss and sleep disturbances have been

mentioned in the sections above. Other metabolic and endocrine effects of HD include cardiac failure, increased peripheral inflammation, and altered endocrine profiles (van der Burg *et al.*, 2009). Neither the pathological mechanisms underlying these nor their impact on the clinical manifestations of the disease are yet fully understood; they are the subject of increasing research interest.

### I. Seizures

Seizures are common in juvenile HD; they are seen in 30–50% of cases (Brackenridge, 1980; Hayden, 1981; Jervis, 1963; Kremer, in press; Osborne *et al.*, 1982). This is in contrast with adult onset HD, in which the incidence of seizures is similar to that of the general population: approximately 1-3% (Hayden, 1981).

A first seizure should not be attributed to HD without further evaluation as it may be indicative of an additional neurological problem, such as a subdural hematoma sustained in a fall. The workup of a first seizure should include a complete examination, laboratory studies to rule out an infection or metabolic disturbance, an EEG, and a brain imaging study. The treatment of a seizure disorder in a person with HD depends on the seizure type. In patients with juvenile HD, myoclonic epilepsy or other generalized seizures are usually treated initially with sodium valproate. Although seizure management in HD is not usually particularly problematic, seizure control can be difficult in some cases and may require a combination of drugs or referral to a specialist.

## III. The Atypical Phenotype, including Juvenile Huntington's Disease

Although the majority of people with HD have the typical hyperkinetic phenotype with symptom onset in adulthood, variant phenotypes exist. Juvenile Huntington's Disease (JHD) is HD which manifests in people before they are 20 years old, and is typically associated with a CAG repeat length of greater than 50. Andrew *et al.* (1993), for example, found that CAG repeat length ranged from 46 to 121 with a median repeat length of 56.5 in a sample of 20 juvenile HD patients. Juvenile HD causes many of the same symptoms as adult-onset disease although the movement disorder is typically hypokinetic rather than hyperkinetic. The presentation of juvenile HD is therefore similar to young-onset Parkinson's disease and levodopa is often used as symptomatic treatment. The rigid of variant of HD is also known as Parkinsonian, akinetic-rigid, or Westphal variant HD, and though it is usually seen in young-onset HD, it can also occur in adults. The overall incidence of this phenotype is around 6–10% of all HD cases (Shoulson and Chase, 1975).

Late onset HD is also well recognized; this is when manifest disease does not develop until an individual is over 60 years old. On average, late onset HD follows a more benign course than typical onset HD, with slower disease progression and, frequently, milder symptoms (Kremer, in press).

## IV. Advanced Disease and End of Life Issues

Patients with advanced HD require significant support in all activities of daily living, usually because of a combination of motor, cognitive, and behavioral symptoms. Communication may be severely limited and muteness is common, often resulting in agitation and frustration due to inability to speak. HD does not cause a global dementia, however, and the ability to recognize and interact with people is frequently relatively well preserved.

As HD progresses, it often becomes increasingly difficult to provide care at home. Looking after someone with advanced HD at home is challenging and frequently exhausting, and carers should be offered considerable levels of support, including periods of respite care. For many people, however, a nursing home is the best option; education and support should be provided for staff to facilitate understanding of the complexity of caring for someone with HD. Periods of respite during which the patient spends time in a nursing home can be a useful way for the patient and his or family to develop a relationship with the nursing home staff; this can ease the transition to full time nursing home residency at a later stage, avoiding or reducing the agitation caused by moving someone with advanced disease directly into an entirely new environment. Involvement of palliative care teams in addition to HD teams can be extremely helpful when managing advanced HD.

As cognitive impairment progresses, patients with HD almost invariably lose the capacity to make decisions about their own care. It is therefore helpful to raise potentially problematic questions early in the course of the disease to allow individuals to plan ahead while they still have the cognitive capacity to do so. Given that people with HD have usually seen the progression of the disease in other family members, bringing up the issues surrounding end of life care rarely comes out of the blue. Topics that commonly arise include:

1. *Advanced Decisions to Refuse Treatment* (previously known as advanced directives): these are extremely helpful and allow individuals to make decisions about their care in advance. They give patients the security of knowing that their wishes will be carried out, even if they are no longer able to make decisions or communicate, and they relieve relatives of the responsibility of making choices.

2. *Power of Attorney*: this allows an individual to nominate someone else to make decisions on their behalf.
3. *Enteral feeding* (usually via percutaneous endoscopic gastrostomy, or PEG): this may be appropriate in patients who are unable to maintain adequate nutrition and body weight.
4. *Use of antibiotics or intravenous fluids* in an individual with end stage disease.

The most common causes of death in people with HD are bronchopneumonia and heart disease, with choking, nutritional deficiencies, and chronic skin ulcers also associated with mortality (Lanska *et al.*, 1988a, 1988b; Sorensen and Fenger, 1992). In our experience, few patients request information about assisted suicide. This remains illegal in the UK.

## V. Looking to the Future: Research into New Treatments for Huntington's Disease

At the time of writing, there is a major drive to find disease-modifying and new symptomatic treatments for HD; many new developments have been made in recent years, and phase 3 trials are ongoing (Novak and Tabrizi, 2010). Much progress has also been made in developing and evaluating sensitive biomarkers which will help to measure the effects of disease modifying therapies in future clinical trials, particularly in the premanifest and early stages of the disease (Paulsen *et al.*, 2008; Tabrizi *et al.*, 2009).

Future disease modifying treatments will, in practice, probably comprise a combination of compounds which will target several key pathogenic pathways to achieve optimal effect. This approach is similar to that used in the treatment of HIV or cancer. Some potential therapeutic strategies are summarized below (Ross and Tabrizi, 2011).

- Enhancing clearance of mutant huntingtin by cellular clearance mechanisms: a number of compounds being tested in mouse models of HD aim to promote clearance of the mutant protein, huntingtin, which is generated by the expanded *HTT* gene.
- Histone deacetylase inhibitors: these target the transcriptional dysregulation that occurs early in HD pathogenesis.
- Inhibitors of proteolytic cleavage of full-length mutant huntingtin to prevent production of the potentially toxic N-terminal fragment.
- Gene silencing: switching off expression of the mutant gene itself.

## VI. Conclusions

HD is a multisystem disease that is characterized primarily by progressive motor, cognitive, and psychiatric symptoms. Management of the disease is

challenging, but there are many options which can ameliorate symptoms and improve quality of life; these are best provided in a collaborative multidisciplinary setting. Extensive research is currently being carried out with the aim of developing treatments that will delay or halt the disease process in the premanifest phase.

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# GENETICS AND NEUROPATHOLOGY OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder that prominently affects the basal ganglia, leading to affective, cognitive, behavioral and motor decline. The basis of HD is a CAG repeat expansion to >35 CAG in a gene that codes for a ubiquitous protein known as huntingtin, resulting in an expanded N-terminal polyglutamine tract. The size of the expansion is correlated with disease severity, with increasing CAG accelerating the age of onset. A variety of possibilities have been proposed as to the mechanism by

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-387003-2.00014-8




0074-7742/10 $35.00

which the mutation causes preferential injury to the basal ganglia. The present chapter provides a basic overview of the genetics and pathology of HD.

## I. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, characterized by affective, cognitive, behavioral, and motor dysfunctions (Albin and Tagle, 1995; Bruyn and Went, 1986; Wilson *et al.*, 1987). HD has a prevalence of 5–10 per 100,000 in South America, North America, Australia, and most European countries and countries of European descent, but significantly lower in Africa and Asia, with an estimated prevalence of 0.5:100,000 in Japan and China, and even lower in South Africa (Walker, 2007). HD affects males and females at the same frequency, and the mean age of onset is around 40 although it can be as early as 4 and as late as 80 years of age. Epidemiologic studies show that in US, there are about 30,000 HD patients and that there are about 150,000 people at risk of developing the disease (Margolis and Ross, 2003; Walker, 2007). The primary site of neuron loss in HD is the striatal part of the basal ganglia, with striatal projection neurons being nearly completely lost in advanced HD. Early dysfunction and late loss of cortical neurons is prominent as well. Neuron loss is progressive, and the dysfunction and loss account for the cognitive and motor decline, leading to death typically about 20 years after onset in adults. The basis of HD is a CAG repeat expansion to >35 CAG in a gene that codes for a ubiquitous protein known as huntingtin, resulting in an abnormally long polyglutamine tract in the protein N-terminus (HDCRG, 1993). Many possibilities have been raised as to the means by which mutant huntingtin results in preferential destruction of the striatum and injury to cortex (Reiner *et al.*, 2003). For example, based on the premise that mutant htt injures neurons in a cell autonomous manner, transcriptional dysregulation (Kegel *et al.*, 2002; Luthi-Carter *et al.*, 2002; Ross, 2002), proteosomal dysfunction (Bence *et al.*, 2001; Chai *et al.*, 1999), induction of autophagy (Kegel *et al.*, 2000, Petersén *et al.*, 2001), release of calcium from intracellular stores (Tang *et al.*, 2009), mitochondrial failure (Bossy-Wetzel *et al.*, 2008), induction of apoptosis (Sanchez *et al.*, 1999; Zuccato *et al.*, 2005), and excitotoxicity at extrasynaptic NMDA receptors (Cowan and Raymond, 2006) have been raised as possible mechanisms responsible for striatal and/or cortical neuron death. Additionally, deficient production and transport of BDNF from cortex to striatum (Cattaneo *et al.*, 2005), excessive cortical release of glutamate, and defective glutamate uptake by glia have been invoked as possible pathogenic mechanisms involving an indirect killing action of mutant htt (Behrens *et al.*, 2002; Cepeda *et al.*, 2007; Joshi *et al.*, 2009; Lievens *et al.*, 2001; Rebec *et al.*, 2006). The present review focuses on the genetics and pathology of HD, with comments on pathogenesis as these relate to findings on HD genetics and pathology.

## II. The HD Gene

The identification of the HD gene relied strongly on the analyses of a large Venezuelan HD kindred with extremely high HD incidence, due to a high frequency of inbreeding. Using standard linkage analyses, the HD gene was mapped to the tip of the short arm of chromosome 4 in 1983 (Gusella *et al.*, 1983, 1994), but it took scientists another 10 years to isolate it and identify the underlying mutation that causes HD (Fig. 1). In 1993, the HD gene was finally identified by The Huntington's Disease Collaborative Research Group (HDCRG), comprising 58 researchers from six independent research groups. Using haplotype analysis of linkage disequilibrium in HD families of distinct ethnicities, they identified a small segment of 4p16.3 as the likely location of the mutation. A new gene, IT-15 (interesting transcript 15), isolated using cloned trapped exons from the target area, was shown to contain a polymorphic trinucleotide CAG repeat within the coding region of the gene that was expanded and unstable on one of the chromosomes of all 75 HD families examined (HDCRG, 1993). The HD locus was found to span 180 kb, consisting of 67 exons, and encoding a protein (huntingtin, htt) of ~350 kDa. Homologues of the human gene have been identified in several species, including but not limited to pig (Matsuyama *et al.*, 2000), mouse (Barnes *et al.*, 1994; Lin *et al.*, 1995), pufferfish (Baxendale *et al.*, 1995), zebrafish (Karlovich *et al.*, 1998), and Drosophila (Li *et al.*, 1999), indicating a conserved essential function of huntingtin through evolution.

The promoter region of the HD gene has features in common with housekeeping genes that are expressed ubiquitously (multiple G/C rich promoter elements and no TATA box sequence (Coles *et al.*, 1998). The CAG repeat (which encodes polyglutamine) is found within exon 1 of all vertebrate HD homologues. Downstream of the CAG repeat is a stretch of polymorphic CCG (polyproline encoding) repeats, also located within exon 1 (HDCRG, 1993). Although highly conserved across different species, with the exception of HEAT motifs, huntingtin has no homology with other proteins (Andrade and Bork, 1995). The function of huntingtin is currently unknown.

The fact that HD shows autosomal dominant inheritance had long been taken to indicate that the HD mutation acts in a "gain-of-function" manner. Discovery of the HD gene allowed further investigation of this notion, leading to several lines of evidence taken to affirm this view (Sharp and Ross, 1996; Ross, 2002). For example, hemizygous inactivation of the HD gene was found to not cause HD symptoms in humans or mice, despite a reduction in HD gene expression to half of normal (Ambrose *et al.*, 1994; Duyao *et al.*, 1995; Nasir *et al.*, 1995; Zeitlin *et al.*, 1995). Moreover, nullizygous mutant mice were found to die *in utero* (Duyao *et al.*, 1995; Nasir *et al.*, 1995; Zeitlin *et al.*, 1995), whereas humans that are homozygous for the HD mutation are born and do not show profound differences from HD heterozygotes in disease onset or progression (Myers *et al.*, 1989; Wexler *et al.*, 1987).

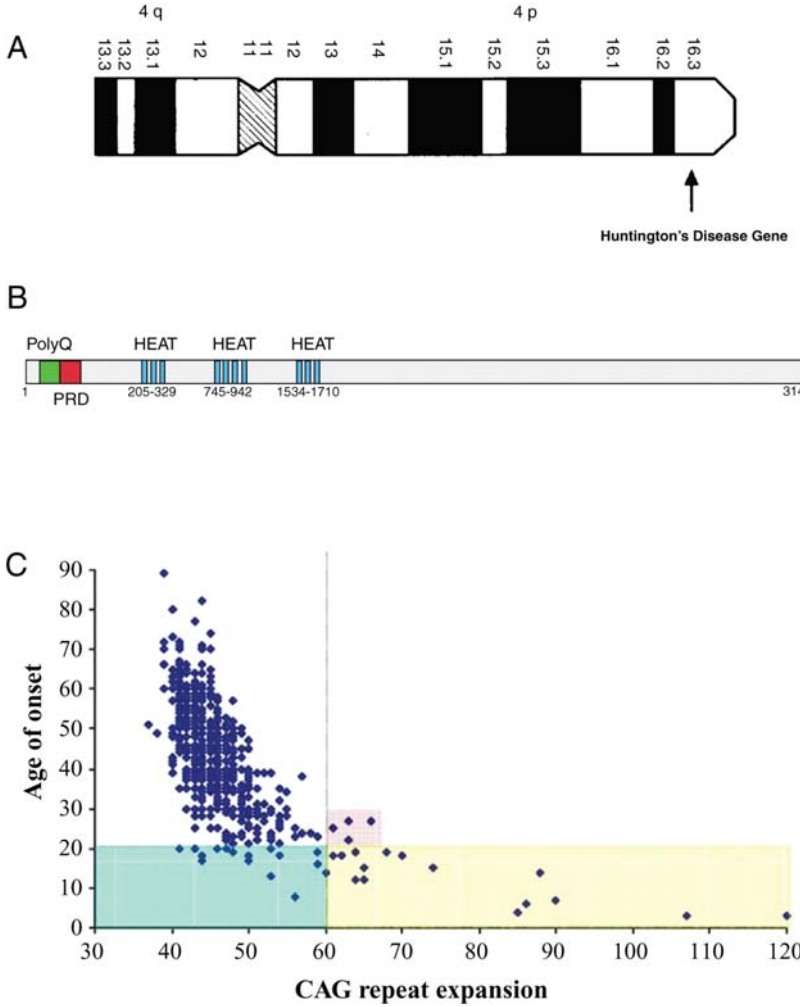


FIG. 1. Image A shows the location of the Huntington's disease gene in band 4p16.3 of chromosome 4 (Adapted from Figure 1 of Gusella *et al.*, 1994). Image B illustrates the huntingtin protein, showing that it contains a polyglutamine region (polyQ) and a proline-rich domain (PRD) at its N-terminus, and 10 HEAT repeats clustered in three domains in the N-terminal half of the protein (Adapted from Figs. 1 and 2 of Harjes and Wanker, 2003). Numbers indicate amino acids. Image C shows a graph depicting the relationship between CAG repeat and Huntington's disease age of onset. Note the overall significant negative correlation between HD onset and the expanded repeat length ($n = 609$, $r^2 = 0.65$, $p = 0.0001$). Nonetheless, the relationship is more complex than this. For example, while there is a strong correlation between CAG repeat and age of onset for adult-onset cases (>20 years) over the 35-55 repeat range, in the case of juvenile (<20 years) onset increasing CAG does not notably advance age of onset highlighted (by dark and pale shading). Moreover, this is also true for the few HD cases found with repeats >200 CAG (not shown in graph). The textured box highlights anomalous adult onset cases with expansion beyond the 60 CAG typically associated with juvenile onset (Adapted from Fig. 4 of Squitieri *et al.*, 2006). (For color version of this figure, the reader is referred to the web version of this book.)

## III. Normal CAG Repeat Length

Early studies by different research groups, involving the analyses of the number of CAG repeats in ~1200 HD individuals and 2000 non-HD individuals, established that the CAG tract in the IT15 gene is polymorphic in the general population, with the normal range of repeat numbers varying from 9 to 11 at the low end and 34–37 at the high end (with an average of 17–20), and that repeat lengths longer than 37 are associated with HD (Read, 1993). Subsequent studies involving large cohorts of individuals who carried between 30 and 40 CAG repeats in the IT15 gene further refined this concept and indicated that repeats up to 35 in length do not cause HD, and that repeat lengths between 36 and 39 are associated with reduced penetrance, meaning that, within this range, some individuals develop HD within their lifetime, while others do not (McNeil *et al.*, 1997; Rubinsztein *et al.*, 1996). Late onset HD with as low as 29 or 34 repeats has, however, been reported (Andrich *et al.*, 2008; Kenney *et al.*, 2006).

## IV. CAG Repeat Length and Disease Onset and Progression

The picture that eventually emerged from numerous studies is that the number of CAG repeats is inversely correlated with age of onset of the disease (Andrew *et èal.*, 1993; Duyao *et al.*, 1993; Snell *et al.*, 1993; Brinkman *et al.*, 1997). Whereas expansions of 40–50 CAG repeats in the mutant HD allele are usually associated with adult onset, juvenile-onset HD, defined as onset before 20 years of age, is usually associated with expansions above 60 CAG repeats (Fig. 1). Clinical manifestations of the disease also differ depending on the length of the CAG tract. The classical HD presentation—adult-onset with predominant chorea—has an onset of around 40 years of age, and the average repeat length is about 44 (Martin and Gusella, 1986; Kremer *et al.*, 1994; Ross *et al.*, 1997; Margolis and Ross, 2003). In patients displaying the reduced-penetrance repeat lengths (36–38 repeats), HD onset not only occurs late in life (60 years of age and older), but patients may present only mild chorea, and without the cognitive, psychiatric and behavioral abnormalities usually associated with longer repeat tracts (McNeil *et al.*, 1997; Rubinsztein *et al.*, 1996). In contrast, chorea is not a major manifestation of juvenile-onset HD, but rigidity and seizures appear to be the predominant characteristics and are often preceded by abnormal behavior (Nance and Myers, 2001; Ribaï *et al.*, 2007). Note, however, that the rare cases with CAG repeats ranging from 60 to >200 indicate that severity does not increase as prominently with repeat expansion beyond 60 (Fig. 1) (Andresen *et al.*, 2006; Squitieri *et al.*, 2006).

## V. CAG Repeat Instability

In the vast majority (>80%) of the hereditary transmissions from HD parents, the expanded repeat is only mildly altered by one or a few CAG repeats, usually decreasing if transmitted maternally, and increasing if transmitted paternally (Bates *et al.*, 1997; Duyao *et al.*, 1993). However, on occasion, paternal transmissions lead to large intergenerational expansions, causing the phenomenon of anticipation, where the age of onset tends to decrease in successive generations (Vonsattel and DiFiglia, 1998). Hence, juvenile-onset HD is associated with paternal transmission in 80–90% of the cases. So far, the longest CAG expansion reported consists of 250 repeats (Nance *et al.*, 1999).

Due to the high rate of meiotic CAG instability during spermatogenesis, normal fathers can also have affected children. Several studies indicate that CAG repeats between 27 and 35 can also be meiotically unstable during paternal transmission, leading to descendents with HD and carrying CAG expansions of 40 or more repeats (Myers, 2004). About 10–15% of all HD cases, in fact, arise from non-affected parents whose repeat lengths fall within the high end of the normal range (Chong *et al.*, 1997; Maat-Kievit *et al.*, 2001; Semaka *et al.*, 2010). Of particular interest, the highest incidence of HD among populations of European descent correlates with the higher frequency of HD alleles bearing 28–35 repeats in these populations compared to populations in either Asia or Africa (Walker, 2007).

The HD CAG repeat is also somatically unstable and undergoes progressive length increases over time. Analyses of tissues from affected individuals showed that repeat mosaicism is present in all tissues, with the greatest levels detected in sperm and in the brain, and in particular in the areas with more pronounced neuropathology (De Rooij *et al.*, 1995; Telenius *et al.*, 1994). Whether this plays a role in pathogenesis is yet uncertain.

## VI. Genetic Modifiers of CAG Repeat Instability

Although paternal transmission has been clearly shown to increase CAG instability, other genetic factors are believed to contribute to CAG instability in HD, including cis-acting factors, such as the size of the CAG tract and HD haplotypes, and trans-acting factors.

Several studies have shown that trinucleotide repeats larger than 28 show instability during replication, and that there is a positive correlation between the instability and the size of the repeat, in particular, in the male germline. Hence, the size of CAG tract is itself a determinant of instability (Leeflang *et al.*, 1999; MacDonald *et al.*, 1993; Wheeler *et al.*, 2007). A very interesting finding is that

postzygotic mechanisms also may play a role in triplet repeat instability in HD (this was first observed in mouse models for HD, Kovtun *et al.*, 2000, 2004). In any event, in maternal transmissions, the daughters will more often carry contractions of the CAG repeat, while the sons will more often carry expansions. While with paternal transmissions expansions are equally frequent in male and female offspring, the CAG repeat increases in length significantly more in sons than in daughters (Wheeler *et al.*, 2007).

In addition, HD haplotypes also appear to influence CAG instability. In a recent study, Warby and collaborators (Warby *et al.*, 2009) found that, in spite of the large number of single nucleotide polymorphisms (SNPs) in the HD gene, disease-associated SNPs form a cluster of similar haplotypes (termed haplogroup A) found in 95% of disease chromosomes. In addition, they found that the same haplogroup is significantly enriched (>80%) in HD genes with intermediate CAG repeats (27–35 CAGs). This finding supports the hypothesis that some variants may have a predisposition for expansion, and that would explain the origin of disease-associated haplotypes.

The availability of mouse models for HD made it possible also to analyze other potential genetic modifiers of CAG repeat instability by assessing the rate of instability in specific gene knockout backgrounds. For instance, somatic CAG instability of transgenic HD mouse models is drastically reduced in mice lacking either the mismatch repair enzyme MSH2 or the base excision repair enzyme OGG1 (Manley *et al.*, 1999; Kovtun *et al.*, 2007). Although the role of Msh2 in CAG repeat expansion is currently not clear, analyses of mice and cell lines lacking OGG1 provided evidence that OGG1 is responsible for initiating an escalating oxidation-excision cycle that leads to progressive age-dependent expansion of the CAG repeats in postmitotic neurons in HD, and possibly in other trinucleotide disorders as well (Kovtun *et al.*, 2007). Thus, at least one mechanism of CAG expansion appears to involve oxidative DNA damage and single-strand break repair.

## VII. Genetic Modifiers of HD Age-of-Onset

Although the primary factor that determines whether and when a person will develop HD is the length of the expanded CAG tract, the precise manifestations of the disease and their onset are clearly affected by modifiers that include environmental and other genetic factors. While it is commonly recognized that the correlation of repeat size accounts for about 70% of the variation in age of onset (Gusella and MacDonald, 2009), there is high variation in age of onset among patients with repeat lengths <55 (Myers, 2004). Strong substantiation that heritable components account for the remaining variation in age of onset was first provided by the HD-MAPS (Modifiers of Age at onset in Pairs of Sibs) study

involving >600 sibling pairs of multiple ethnicities (Djoussé *et al.*, 2003; Li *et al.*, 2003). These studies and their follow-ups provided strong evidence of linkage between chromosome 6q and 4q to age of onset of neurological symptoms (Li *et al.*, 2006). Analyses of HD Venezuelan kindreds, encompassing >15,000 individuals and comprising 4500 sibships, also confirmed the association of several loci with age of onset and identified significant linkage to chromosomes 2p and 6q, among others (Gayán *et al.*, 2008; Wexler *et al.*, 2004). However, in both cases, the genomic regions are large and so far the specific modifier genes have not been identified. Genome-wide studies using densely spaced single-nucleotide-polymorphisms (SNPS) are currently been applied in an expanded version of the HD-MAPS collaboration to identify the modifier genes in these regions (Gusella and MacDonald, 2009).

The search for genetic modifiers among genes that are connected to pathways and processes thought to be involved in HD also led to the identification of additional candidates. GRIK2 (glutamate receptor ionotropic kainate2, also known as GLUR6) was the earliest reported genetic modifier, and multiple studies have shown that a polymorphic TAA trinucleotide repeat in its 3' untranslated region (3'UTR) is associated with earlier HD onset (Gusella and MacDonald, 2009; MacDonald *et al.*, 1999; Rubinsztein *et al.*, 1997). The mechanism by which different GRIK2 alleles affect onset is still unknown.

Polymorphisms in huntingtin-associated protein 1 (HAP1) and Atg7 (autophagy-related 7 homolog) genes have also been shown to play a role in onset age in HD. By sequencing the HAP1 gene in unaffected populations, six polymorphisms have been identified, including one that substitutes methionine (M441) for threonine (T441) at amino acid 441. Analyses of 980 European HD patients revealed that patients homozygous for the HAP1 M441 genotype (that substitutes threonine by methionine) showed an 8-year delay in the onset. Functional assays demonstrated that human M441-HAP1 interacts with mutant htt more tightly than does human T441-HAP1 and protects against mutant htt-induced toxicity (Metzger *et al.*, 2008). Using the same approach, the same group reported one polymorphism in the Atg7 gene that substitutes alanine for valine (V471A). This polymorphism showed a significant effect and was associated with an earlier disease onset of 4 years. Although the mechanism by which this polymorphism affects age of onset is unknown, it has been hypothesized that the V471A Atg7 has reduced autophagic function (Metzger *et al.*, 2010).

The hypothesis that somatic instability of the HD CAG repeat is itself a modifier of disease age of onset gained support by the finding that somatic instability is a significant predictor of onset age, with larger repeat length gains associated with earlier disease onset (Swami *et al.*, 2009; Veitch *et al.*, 2007). Hence, factors that are involved in the control of repeat instability may also represent potential genetic modifiers for age of onset.

Analyses of animal models for HD also implicate several other genes as potential genetic modifiers of age of onset. For instance, age of onset is significantly

earlier and pathology is exacerbated in mouse models of HD lacking either the heat shock protein Hsp70 (Wacker *et al.*, 2009) or the neurotrophin BDNF (Canals *et al.*, 2004), while inhibition of caspase-1 delays both the age of onset of motor symptoms and the occurrence of other behavioral and neuropathological changes (Ona *et al.*, 1999). The role of any of these genetic factors in HD in humans, however, remains to be verified.

## VIII. HD: A True Dominant Gain-of-Function Disorder?

HD is one of a group of inherited neurodegenerative disorders, commonly referred to as "trinucleotide repeat disorders," caused by expansions of trinucleotide repeats in distinct genes. In at least nine of these diseases, including HD, these expansions involve CAG repeats that are present in the coding region of the gene and are translated into polyglutamine stretches. Although the mutant protein of the distinct disorders do not share any homology or sequence similarity, except for the presence of the polyglutamine tract, all of them have similar features (for example, repeat length—onset age correlation, and dominant inheritance) and are likely to possess some similarities in their pathogenic mechanisms. Since neuronal degeneration occurs in different areas of the brain in these different CAG repeat diseases, there clearly are also disease mechanisms specific to each disorder that impart the differential regional vulnerability. The dominant pattern of inheritance of HD strongly indicates that HD, like all other polyglutamine disorders, is caused by a gain-of-function mechanism and that the expanded polyglutamine stretch is responsible for the pathogenesis.

Homozygous HD patients are rare, and there is still controversy over whether homozygosity for the mutation in HD is associated with a more severe phenotype. Most information on homozygosity in HD has come from analyses of probable homozygous offspring within the Venezuelan kindreds (Wexler *et al.*, 1987) or from other sporadic cases in which both parents are affected (Alonso *et al.*, 2002; Dürr *et al.*, 1999; Myers *et al.*, 1989). In all these reports, the age-at-onset appeared similar in homozygotes and heterozygotes, and both progression and severity of the disease were in some cases actually worse in the heterozygotes. Together, these reports led to the conclusion that HD displays complete dominance. However, this conclusion was based on clinical evaluation of eight potential homozygous and only two confirmed cases, and did not take into account differences in CAG tract sizes between siblings, or other possible genetic modifiers. In contrast, a more detailed comparison between a large homozygous patients' series and their heterozygous counterparts in a multicenter study revealed significant clinical and neuropathological differences between the two groups (Squitieri *et al.*, 2003). In this study, not only the disease progression was more rapid in homozygous patients, but also homozygous patients appeared to have a wider spectrum of

neurological symptoms. More recent work involving cell lines derived from heterozygous and homozygous HD patients (Mormone *et al.*, 2006; Squitieri *et al.*, 2010; Varani *et al.*, 2003) and analyses of mouse models for HD (Fossale *et al.*, 2002; Graham *et al.*, 2006; Lin *et al.*, 2001) also support the notion that HD is more severe in homozygosity. Thus, more recent work is consistent with the notion that, like other triplet repeat disorders, HD is not a true dominant disorder, and that gain of function is only one of the facets of this devastating disease.

## IX. Expression of Huntingtin in Normal and HD Human brain

Huntingtin mRNA and protein are widely distributed in mammalian brain, and almost no brain region is devoid of huntingtin-containing perikarya—although glial cells typically show only low levels (Bhide *et al.*, 1996; Fusco *et al.*, 1999; Gutekunst *et al.*, 1995; Landwehrmeyer *et al.*, 1995; Li *et al.*, 1993; Sapp *et al.*, 1997; Sharp and Ross, 1996; Strong *et al.*, 1993; Vonsattel and DiFiglia, 1998). Large neuronal perikarya tend to be richer in huntingtin than medium-sized or small neuronal perikarya, and huntingtin-positive neurons are especially abundant in the telencephalon and thalamus, but seemingly sparse in the hypothalamus. Within telencephalon, the highest density of huntingtin-rich neurons is in cerebral cortex, in which pyramidal neurons of layers 3 and 5 are especially rich (Fig. 2), and in hippocampus, in which the pyramidal neurons of CA2–CA3 are labeled intensely for huntingtin. The vast majority of striatal projection neurons are, however, only moderate in huntingtin, but scattered large neurons in striatum and the large neurons of globus pallidus externus, the ventral pallidum, basal nucleus of Meynert, and the globus pallidus internus are rich (Fig. 2) (Bhide *et al.*, 1996; Fusco *et al.*, 1999; Gutekunst *et al.*, 1995; Landwehrmeyer *et al.*, 1995). The disease-producing mutation in the HD gene does not appear to affect its regional expression in brain (Bhide *et al.*, 1996; Gourfinkel-An *et al.*, 1997; Landwehrmeyer *et al.*, 1995; Sapp *et al.*, 1997; Schilling *et al.*, 1995; Vonsattel and DiFiglia, 1998). Thus, while the widespread distribution of huntingtin in brain indicates that it possesses a role in the functioning of many brain neurons, this function is not limited to the brain regions and neurons that are the major target of HD, and huntingtin expression is not obviously selectively impaired in the regions or neuron types most affected by the HD mutation. At the cellular level, huntingtin is found in the cytoplasm of neuronal perikarya, in dendrites, and to seemingly a lesser extent in axons and terminals (Vonsattel and DiFiglia, 1998). Ko *et al.* (2001) recently suggested, based on studies using antibodies directed against different epitopes of wild-type Htt, that Htt may play diverse roles in cellular function. Presumably as a reflection of this diversity, they found that different epitopes of huntingtin are immunohistochemically detectible in different subcellular

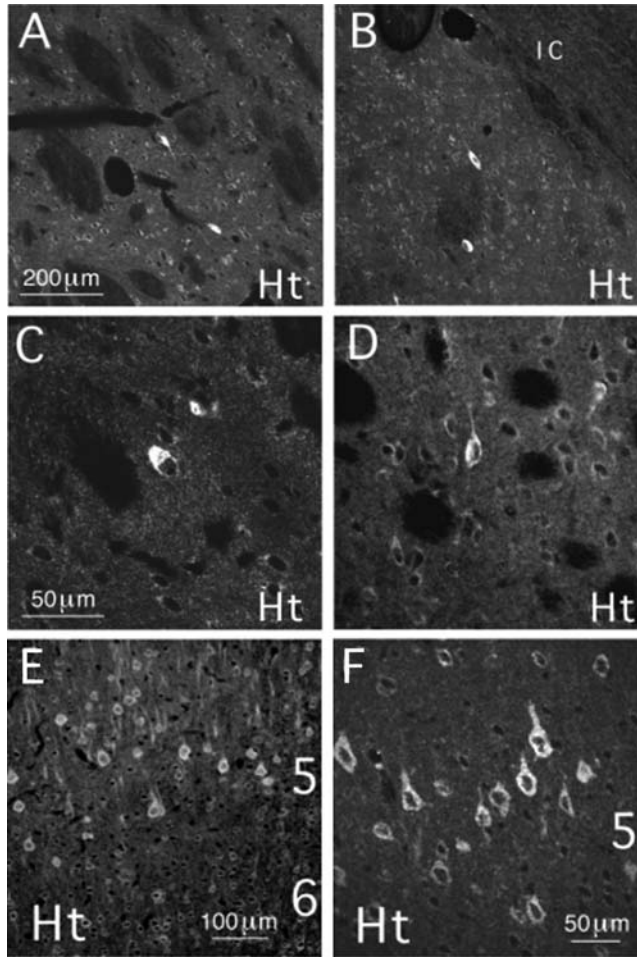


FIG. 2. Immunofluorescence labeling for huntingtin (*Ht*) in the rat striatum viewed with CLSM. Two low-magnification fields (*A*, *B*) and two high-magnification fields (*C*, *D*) show that scattered large neurons intensely labeled for huntingtin and numerous medium-sized neurons moderately labeled for huntingtin are present in striatum. Magnification in *A* is as in *B*; magnification in *C* is as in *D*. Images E and F show immunofluorescence labeling for huntingtin in the lower layers of rat cerebral cortex, at increasingly higher magnification. Both fields show intense labeling of pyramidal neurons in Layer 5 of cortex. All images are from Fusco *et al.* (1999).

compartments, implying differential processing or folding of Htt for its role in the different compartments. Among its functions, huntingtin appears to be a cell membrane-associated scaffolding protein involved in vesicular trafficking (DiFiglia *et al.*, 1995; Qin *et al.*, 2004; Sharp *et al.*, 1995; Velier *et al.*, 1998; Wood *et al.*, 1996). Immunolabeling and immunoprecipitation studies indicate

that huntingtin may also be involved in the endosomal-lysosomal protein degradation pathway (DiFiglia *et al.*, 1995; Gutekunst *et al.*, 1995; Sapp *et al.*, 1997; Sharp *et al.*, 1995; Velier *et al.*, 1998; Vonsattel and DiFiglia, 1998; Wood *et al.*, 1996). Nuclear localization of full-length wild-type Htt has also been reported (Atwal *et al.*, 2007; Dorsman *et al.*, 1999; Wilkinson *et al.*, 1999).

Neuropathological studies suggest that the pathogenic HD gain of function could be the formation of ubiquitinated aggregates of the N-terminal fragment of mutated huntingtin, which is thought to occur due to enhanced cleavage and aggregation of the polyglutamine rich part of the mutant huntingtin N-terminus (DiFiglia *et al.*, 1997; Gutekunst *et al.*, 1999; Li and Li, 1998; Maat-Schieman *et al.*, 1999; Martindale *et al.*, 1998; Sieradzan *et al.*, 1999; Vonsattel, 2008). Aggregates of mutant protein are observed in neocortex, entorhinal cortex, subiculum, hippocampal pydamidal neurons, and striatum, more so in advanced and/or juvenile onset HD (Fig. 3). Aggregates are, however, rare in globus pallidus, substantia nigra, and cerebellum. Some aggregates in HD brain possess an amyloid-like structure, suggesting parallels in aggregate formation with other amyloid-associated diseases such as Alzheimer's and prion diseases (McGowan *et al.*, 2000). Both cytoplasmic and intranuclear aggregation have been observed in HD brain, the latter termed neuronal intranuclear inclusions, or NIIs (Kuemmerle *et al.*, 1999). While considerable attention has been given to the possibility that these aggregates are themselves pathogenic (Davies *et al.*, 1997; DiFiglia *et al.*, 1997; Kim and Tanzi, 1998; Saudou *et al.*, 1998; Sisodia, 1998), the means by which they might lead to neuronal death remains uncertain (Cha *et al.*, 1998; Hackham *et al.*, 1998a,b; Sisodia, 1998). Mutant huntingtin aggregates may, in part, be pathogenic by their capacity to incorporate and thus sequester vital proteins such as the transcription factor TATA-binding protein (van Roon-Mom *et al.*, 2002). The possibility that the aggregates may, at least in part, act by inactivating both mutant and normal huntingtin has been raised by recent evidence showing that the aggregates which form in HD can sequester normal-length polyglutamine-containing proteins, including Htt and CREB-binding protein, both of which promote BDNF production (Cattaneo *et al.*, 2001; Narain *et al.*, 1999; Nucifora *et al.*, 2001; Ona *et al.*, 1999; Preisinger *et al.*, 1999; Shieh *et al.*, 1998; Tao *et al.*, 1998; Wheeler *et al.*, 2000). Neuropathological studies, however, show that formation of NIIs in HD victims is not prominent in cerebral cortex until advanced stages of HD and is never prominent in striatum (1–4% of neurons) at any stage (DiFiglia *et al.*, 1997; Gutekunst *et al.*, 1999; Kuemmerle *et al.*, 1999; Sapp *et al.*, 1999). In fact, the striatal neurons that do possess NIIs tend to be interneurons, which survive well in HD, rather than projection neurons (Kuemmerle *et al.*, 1999). This brings into question if NIIs are pathogenic. Neuropil aggregates (found in spines, dendrites, and axons) are far more common in HD cortex and striatum than NIIs, and thus may be pathogenic by interfering with neuronal function, particularly corticostriatal communication (DiFiglia *et al.*, 1997; Gutekunst *et al.*, 1999; Kuemmerle *et al.*, 1999; Sapp *et al.*, 1999). Regardless of the motor versus mood symptoms, there is a consistently higher number of

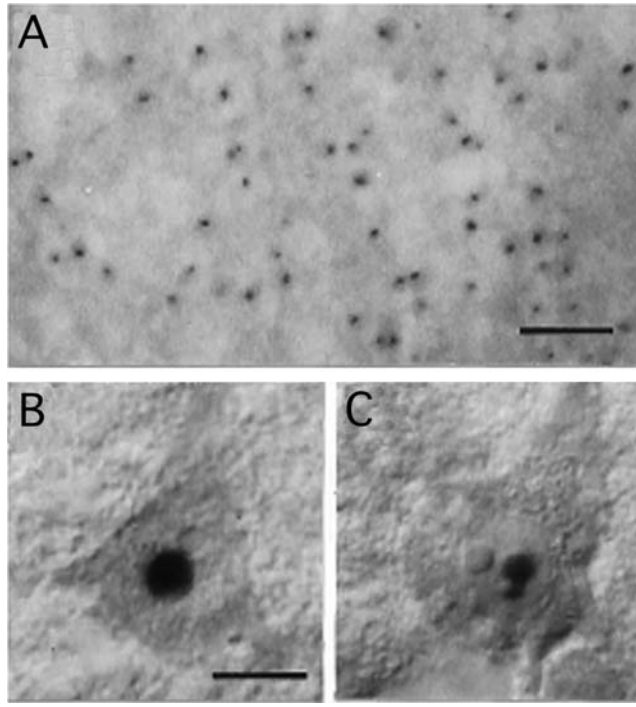


FIG. 3. Images showing immunolabeling for huntingtin in HD brain, revealing aggregates of mutant huntingtin in neuronal nuclei, termed intranuclear inclusions (NIIs). Image A shows the presence of numerous NIIs in cerebral cortex of juvenile HD victim at low magnification. Images B and C show immunolabeled NIIs in individual cortical pyramidal neurons in the same juvenile HD victim, using Nomarski optics to highlight the NIIs. The nucleolus in each cell is unlabeled. These images are adapted from A-C of Fig. 1 from DiFiglia *et al.* (1997).

aggregates in the superior frontal gyrus than in the motor cortex, suggesting a consistent regional difference in aggregate density that thus does not account for differing symptomatology between cases (van Roon-Mom *et al.*, 2006).

### X. HD Brain Pathology and the Vonsattel Grading System

Neuropathological and imaging studies reinforce the view that brain abnormalities in HD develop well before evident symptoms, are progressive, and eventually involve the entire brain to a greater or lesser extent, resulting in about 25% brain weight loss in advanced HD (Halliday *et al.*, 1998; Sharp and Ross, 1996). Nonetheless, the most prominent neuropathology in HD occurs within the striatal part of the basal ganglia, in which gross atrophy is accompanied by extensive

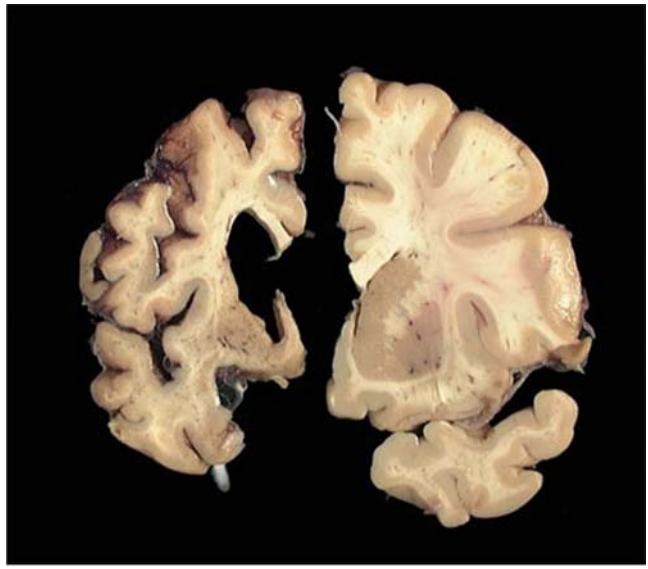

FIG. 4. Coronal slices though human telencephalon, showing a normal brain on the right and an advanced HD brain (Grade 4) on the left. Note the profound shrinkage of cortex and caudate and the resulting ventricular expansion in the HD brain. Image courtesy of the Harvard Brain Tissue Resource Center. (For color version of this figure, the reader is referred to the web version of this book.)

neuronal loss and astrogliosis, both of which become more severe as the disease progresses, with the atrophy leading to great enlargement of the lateral ventricles (Fig. 4). At least some of these dying neurons show nuclear fragmentation and marker expression characteristic of apoptotic cell death (Thomas *et al.*, 1995; Vis *et al.*, 2005). Reactive astrocytes are increased in HD striatum and show increased coupling by gap junctions, which may provide increased spatial buffering in an attempt to maintain a beneficial environment for neurons (Vis *et al.*, 1998). Striatal pathology in both caudate and putamen is more prominent caudally than rostrally in early disease, and striatal degeneration proceeds, for unknown reasons, in a dorsomedial to ventrolateral direction (Roos *et al.*, 1985; Vonsattel, 2008). Caudate atrophy as detected by MRI or CT has been shown to be correlated with CAG repeats and with a worsening of the UHDRS motor score (Culjkovic *et al.*, 1999; Jech *et al.*, 2007). Marked neuronal loss and shrinkage is also seen in deep layers of the cerebral cortex. Other regions, including globus pallidus, hippocampus, amygdala, thalamus, subthalamic nucleus, substantia nigra, and cerebellum, show varying degrees of atrophy and/or neuronal loss, depending on disease stage (Rosas *et al.*, 2003). The neuron loss is reflected in regional brain atrophy. For example, late in disease, volumetric losses of the following magnitudes are observed: 20% in cortex, 30% in cerebral white matter, 60% in striatum, 55%

in globus pallidus, and 30% in thalamus (de la Monte *et al.*, 1988; Lange *et al.*, 1976; Heinsen *et al.*, 1994). Caudate shrinkage is significant already 10 years from estimated disease onset, while putamen and globus pallidus shrinkage is not significant until 3 years before estimated disease onset (Aylward *et al.*, 1996). Gene expression analysis of caudate, cerebellum, prefrontal association cortex, and primary motor cortex shows the greatest number and magnitude of differentially expressed mRNAs in caudate, followed by motor cortex, then cerebellum, with no detected changes in prefrontal cortex (Hodges *et al.*, 2006). Thus, caudate is most affected in HD, and cerebral cortex is not uniform in its response in HD. Note that caudate volume loss, overall brain volume loss, and white matter disorganization are manifest early in HD, and these HD brain abnormalities precede overt signs of disease (Aylward *et al.*, 1994; Kassubek *et al.*, 2004c; Paulsen *et al.*, 2006; Squitieri *et al.*, 2009; Reading *et al.*, 2005; Rosas *et al.*, 2005).

A system for grading HD neuropathological severity has been developed based on macroscopic and microscopic criteria related to striatal morphology (Fig. 5) (Vonsattel *et al.*, 1985). This system recognizes five Grades (0–4) designated in the ascending order of severity, with the grades correlating closely with the degree of clinical disability. There are no evident gross, and few microscopic abnormalities in premanifest HD striatum (Grade 0, also termed presymptomatic). The microscopic abnormalities that can be present involve increased abundance of oligodendrocytes and neurons with nuclear aggregates in the tail of caudate, and some neuron loss in head of caudate (Gómez-Tortosa *et al.*, 2001; Vonsattel, 2008). Grade 1 cases have abnormalities that can be detected microscopically in striatum (50% neuron loss in head of caudate) but gross atrophy is not evident, as the ventricular profile of the caudate maintains its normal convex appearance. The Grade 1 changes involve neuron loss and gliosis in the medial paraventricular portions of the caudate, in the tail of the caudate, and in the dorsal part of the putamen. In Grade 2, striatal atrophy is present, but the ventricular profile of the caudate remains convex, but less so than in normal brain. The lateral half of the striatum shows relative preservation in Grades 1–2. In Grade 3, striatal atrophy is more severe, and the ventricular profile of the caudate is flat. In Grade 4, 95% of caudate neurons are lost, striatal atrophy is severe, and the ventricular surface of the caudate is concave. Astrocytes are greatly increased above normal in HD Grades 2–4. This grading system has come to be widely used in neuropathological studies of HD that seek to describe changes as disease progresses.

## XI. Basal Ganglia Pathology in HD

The major site of pathology in HD is the basal ganglia, which consists of striatal and pallidal subdivisions. The striatum consists of two major neuron types,

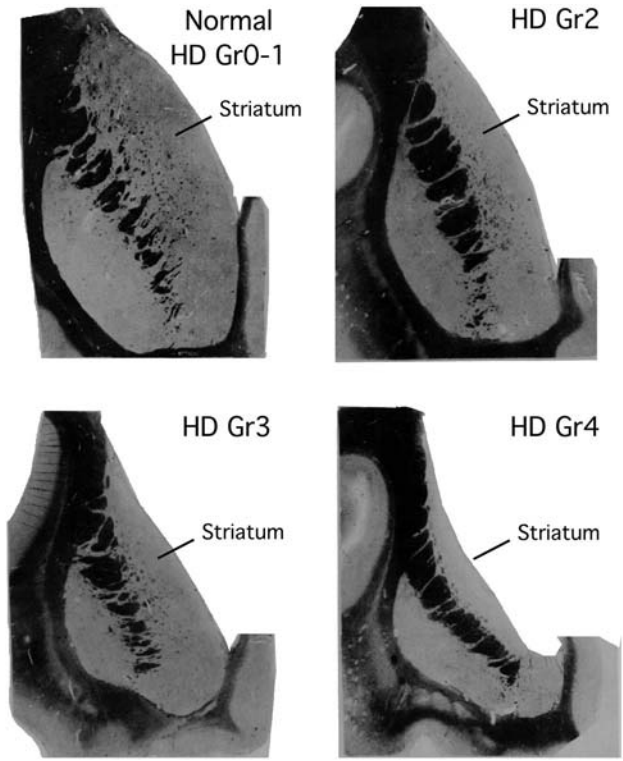


FIG. 5. Schematic illustrations of caudate at HD Grades 0 through 4 according to the Vonsattel et al grading scale. Note that the ventricular profile of the caudate is diagnostic for classification, and the extent of caudate neuron loss distinguishes normal from HD, and Grade 0 versus Grade 1 HD. This illustration is adapted from Fig. 2 of Vonsattel *et al.* (1985).

projection neurons and interneurons, while globus pallidus consist mainly of projection neurons. We will detail how HD affects these various neuronal populations below. Of note, striatal neuron loss in HD largely involves projection neurons, with most striatal interneuron types highly resistant to HD.

### A. Striatum—Projection Neurons

Striatal projection neurons are all GABAergic and can be subdivided into four major types based on their primary projection target: (1) those projecting only or mainly to the external segment of globus pallidus (GPe), which are typically rich in enkephalin (ENK) and poor in or devoid of substance P (SP), and located in the

striatal matrix compartment; (2) those projecting mainly to the internal segment of globus pallidus (GPi), which are rich in SP and dynorphin (DYN) but poor in ENK, and located in the striatal matrix compartment; (3) those projecting mainly to the substantia nigra pars reticulata (SNr), which are also rich in SP and DYN, and typically poor in ENK, and located in the striatal matrix compartment; and (4) those projecting to the substantia nigra pars compacta (SNc), which also are rich in SP and DYN, and typically poor in ENK, and largely localized to the striatal patch compartment (Beckstead and Cruz, 1986; Feger and Crossman, 1984; Kawaguchi *et al.*, 1990; Parent *et al.*, 1989, 1995; Reiner and Anderson, 1990; Reiner *et al.*, 1999; Wu *et al.*, 2000). Because these striatal neurons are GABAergic, they all express the enzymes that convert glutamate to GABA, namely the 65kD and 67kD forms of glutamic acid decarboxylase (GAD). The perikarya of striato-GPe neurons and their terminals in GPe are also enriched in D2 dopamine and A2a adenosine receptors (Fink *et al.*, 1992; Le Moine and Bloch, 1995; Schiffmann *et al.*, 1991). In turn, the perikarya of striato-GPi neurons and their terminals in GPi are enriched in D1 dopamine receptors, as are the perikarya of striatonigral neurons and their terminals in substantia nigra (Fink *et al.*, 1992; Le Moine and Bloch, 1995; Schiffmann *et al.*, 1991). All striatal projection neuron perikarya and terminals also possess cannabinoid receptors (Glass *et al.*, 1997; Herkenham *et al.*, 1991; Mailleux and Vanderhaeghen, 1992). These various neurochemical traits provide markers by which the progressive effect of HD on these projection neuron populations can be characterized, either by studying the loss of terminals in the target areas or by studying loss of the perikarya. These four neuronal types play different roles in movement control, and it is thus valuable to characterize how HD affects them to better understand HD pathophysiology. As summarized below, the overall data indicate that while striatal projection neurons as a class are highly vulnerable in HD, and as a result projection neurons markers are lost from striatum as disease progresses (Goto *et al.*, 1989; Seto-Ohshima *et al.*, 1988), projection neuron types do exhibit differences in susceptibility. Notably, striato-GPe and striato-nigral neurons are lost more rapidly in HD than are striato-GPi neurons.

Immunohistochemical studies have indicated that ENK/GAD+ terminals in GPe and SP/GAD+ terminals in the substantia nigra are lost sooner in HD progression than are SP/GAD+ terminals in GPi (Figs. 6–8). For example, depletion of ENK+ immunostaining from GPe has been noted in premanifest HD (Albin *et al.*, 1990b, 1992; Hedreen and Folstein, 1995), and striatal PPE expression appears reduced in premanifest HD (Albin *et al.*, 1991; Augood *et al.*, 1996, 1997). By Grade 1, ENK/GAD+ fibers in GPe are reduced to about 35% of control abundance and SP/GAD+ fibers in SNc and SNr are reduced to about 30% and 50%, respectively, of control abundance (Allen *et al.*, 2009; Deng *et al.*, 2004; Sapp *et al.*, 1995). By contrast, the loss of striatal terminals in GPi is much less in Grade 1, with SP/GAD+ fibers being 70–80% of control abundance (Deng *et al.*, 2004; Sapp *et al.*, 1995). The loss of striato-GPe and striato-nigral projections remains

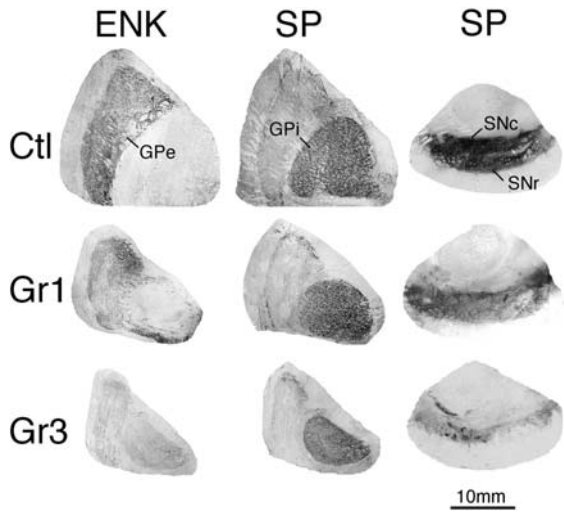


FIG. 6. Images of immunohistochemically labeled sections showing GPi, GPe, and substantia nigra in control, Grade 1 HD, and Grade 3 HD cases, immunostained for SP in the case of GPi and the nigra and for ENK in the case of GPe. In the control, SP+ fibers abound in GPi, ENK+ fibers abound in GPe, and SP+ fibers abound in the nigra. In Grade 1 HD, ENK+ fibers in GPe and SP+ fibers in the nigra are depleted, while SP+ fibers in GPi remain abundant. The contrast is even more evident in the Grade 3 specimen, where ENK+ fibers are markedly depleted in the atrophied GPe and SP+ fibers in the nigra are sparse and patchy, but SP+ fibers in GPi are still quite prominent. This illustration is Fig. 5 from Deng *et al.* (2004).

greater than the loss of striato-GPi projections through Grades 2 and 3 (Albin *et al.*, 1990a; Allen *et al.*, 2009; Deng *et al.*, 2004; Reiner *et al.*, 1988; Sapp *et al.*, 1995). For example, in Grade 2, striatal terminals in GPe are at 25% of normal abundance (Deng *et al.*, 2004; Sapp *et al.*, 1995), and in SNc and SNr are at about 35% of normal abundance (Deng *et al.*, 2004). By contrast immunolabeled striatal terminals in GPi are at 60% of their normal abundance (Deng *et al.*, 2004; Sapp *et al.*, 1995). In Grade 3 HD, immunolabeled striatal fibers in GPe, SNc, and SNr are at 20% of normal abundance, but in GPi are at 50% of normal abundance (Deng *et al.*, 2004). By Grade 4 of HD, however, profound loss in all projection systems is apparent (Albin *et al.*, 1990a; Reiner *et al.*, 1988), with striato-GPe and striato-GPi projections at about 5% of normal, and striato-SNc and SNr projections at 10% of normal (Deng *et al.*, 2004). Thus, striato-GPe and striatonigral neurons appear to be lost more rapidly than striato-GPi neurons during HD progression. DTI confirms massive loss of striatal projections in HD, indicating the immunolabeling changes reflect real fiber loss and not just staining loss (Douaud *et al.*, 2009). Direct support for this premise at the perikaryal level has come from *in situ* hybridization histochemistry for SP and ENK mRNA in HD striatum (Albin *et al.*, 1991; Richfield *et al.*, 1995a, 1995b), and from binding of D1 and D2 dopamine (Glass

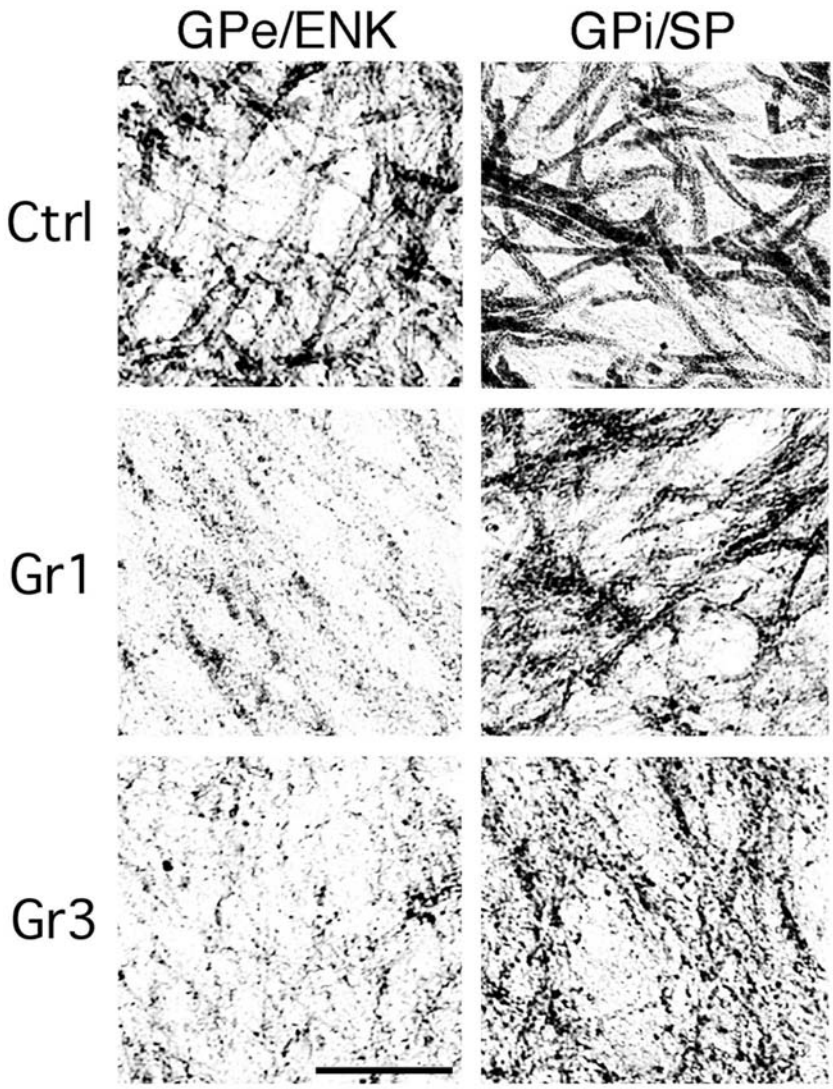


FIG. 7. High-power images showing SP+ fibers in GPi (B, D, F) and ENK+ fibers (A, C, D) in GPe. In the control case, abundant woolly fibers can be seen in both GPi and GPe. In Grade 1, loss of ENK+ fibers in GPe is apparent, while the SP+ fibers in GPi are indistinguishable from that in control. In Grade 3, ENK+ woolly fibers are completely absent, while the SP+ fibers in GPi are relatively preserved, although a decrease in terminal density is apparent. This illustration is Fig. 6 from Deng *et al.* (2004).

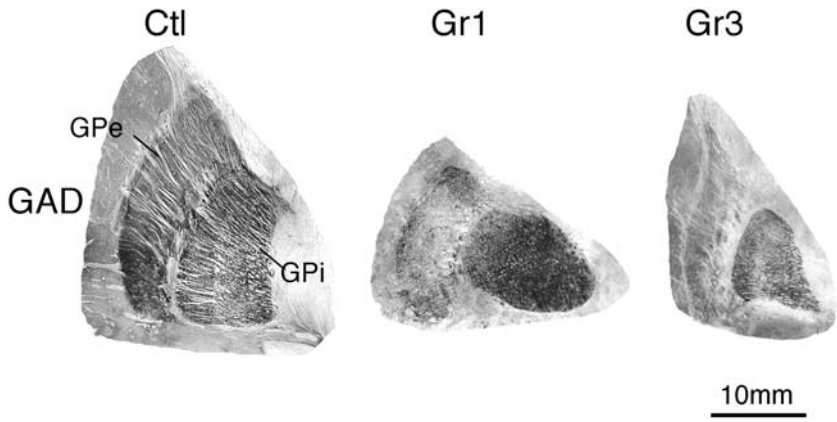


FIG. 8. Low-power images showing GAD+ staining in both GPi and GPe of control, Grade 1 HD, and Grade 3 HD cases. Note the greater loss of GAD+ woolly fibers from GPe than from GPi. This illustration is Fig. 7 from Deng *et al.* (2004).

*et al.*, 2000) and A2a adenosine receptors (Glass *et al.*, 2000) in HD striatum. For example, the loss of the SP+ projection to nigra and the loss of the ENK+ projection to GPe, with the relative preservation of the SP+ projection to GPi, predict that SP+ neuron survival should be better than ENK neuron survival in HD striatum. In fact, neurons expressing mRNA for the SP precursor (i.e., preprotachykinin or PPT) are more abundant in striatum during Grades 1–3 HD than are neurons expressing mRNA for the ENK precursor PPE (Richfield *et al.*, 1995a, 1995b).

These findings for striato-GPe and striato-GPi projections in HD are also compatible with the radioimmunoassay (RIA) study of Seizinger *et al.* (1986), who reported that dynorphin (DYN), which is co-localized with SP in striatal terminals in GPi (Reiner *et al.*, 1999), was undiminished in GPi in HD victims. By contrast, the PPE-derived neuropeptide MERGL was only half its normal abundance in GPe in the HD brains they studied. Biochemical studies have also shown that GABA and GAD are more greatly decreased in GPe than in GPi in symptomatic HD (Ellison *et al.*, 1987; Spokes, 1980; Storey and Beal, 1993). Since striato-GPi and striato-GPe projection neurons are both GABAergic (Reiner and Anderson, 1990), these results too indicate a preferential loss among striatopallidal neurons of those projecting to GPe. One prior biochemical study has suggested that GABA is diminished in GPe in premanifest HD while GABA in GPi remains normal (Reynolds and Pearson, 1990).

Biochemical studies of SP, DYN, GABA, or GAD also indicate that striatal input to nigra is severely depleted in HD (Buck *et al.*, 1981; Beal *et al.*, 1988; Ellison *et al.*, 1987; Emson *et al.*, 1980; Gale *et al.*, 1977; Kanazawa *et al.*, 1977, 1979;

Seizinger *et al.*, 1986; Spokes, 1980; Spokes *et al.*, 1980; Storey and Beal, 1993). Of note, Seizinger *et al.* (1986) found that DYN in nigra and MERGL in GPe were halved in HD victims, but DYN in GPi was undiminished. The possibility that the striatal projection to SNc is differently affected in HD than that to SNr has been of interest because they arise from different striatal neuron types, and because Hedreen and Folstein (1995) reported that striosomal neurons, whose principal projection target is pars compacta (Gerfen, 1992), are already affected at Grade 0. Judging whether the SP+ fiber loss is greater for SNc than for SNr is difficult, however, because many dopaminergic neurons of SNc in primates are dispersed within the SNr territory, making it ambiguous to precisely define the boundaries of SNc (Arsenault *et al.*, 1988; Hökfelt *et al.*, 1984). Not surprisingly, the available immunolabeling data do not unambiguously support the notion that presymptomatic HD is characterized by loss in the striato-SNc projection but not in the striato-SNr projection (Deng *et al.*, 2004). Similarly, by RIA Beal *et al.* (1988) observed extensive loss of SP from both SNr and SNc by Grade 1, followed by further loss in subsequent grades, with no clear differences between them at any grade. Other biochemical studies have reported varied results, however, with some observing greater loss of SP or GABA from SNr than SNc (Buck *et al.*, 1981; Ellison *et al.*, 1987; Emson *et al.*, 1980; Kanazawa *et al.*, 1977), and others the opposite (Gale *et al.*, 1977). One study that distinguished HD cases as choreic (early to mid-HD) versus rigid (late HD) reported greater loss of GAD from SNr than SNc in both (Spokes, 1980). Tippett *et al.* (2007) have reported that preferential striosomal loss (i.e., striato-SNc neuron loss) is not invariably a trait of early HD but does appear associated with mood abnormality when it does occur.

The major findings in HD obtained using neuropeptides or GAD as markers have been confirmed by studies using additional markers of striatal neurons and their terminals. For example, Grade 0 HD has been found to be characterized by loss of cannabinoid, D2 and A2a receptor binding from striatum and by a large increase in GABAA binding in GPe (Glass *et al.*, 2000). These findings are consistent with a preferential loss of ENK+ input to GPe at Grade 0. The absence of reductions in D1 receptor binding in striatum or in GPi at Grade 0 (Glass *et al.*, 2000) suggests that striatal SP+ neurons in general and those projecting to GPi, in particular, are largely unaffected in premanifest HD. The occurrence of reduced D1 receptor binding in SNr at Grade 0 (Glass *et al.*, 2000) and reduced striatal message for D1 receptors and PPT at Grade 0, however, suggest that defects not yet evident at the peptide level or the level of GABA/GAD production are present in presymptomatic HD in striato-SNr projection neurons.

Grade 1 HD is characterized by about 90% loss of striatal D2 dopamine and A2a adenosine receptors (localized to ENK+ neurons), 75% loss of striatal cannabinoid receptors, 50% loss of striatal D1 receptors, near complete depletion of D2 and A2a adenosine receptors from GPe, continued upregulation of GABAA receptor binding in GPe, complete preservation of D1 receptors in GPi, greater

preservation of cannabinoid receptors in GPi than GPe or SNr, and 20% loss of D1 receptors from SNr (Allen *et al.*, 2009; Glass *et al.*, 2000; Richfield and Herkenham, 1994; Walker *et al.*, 1984). These findings are consistent with relative preservation of the striato-GPi projection at Grade 1 concomitant with considerable loss in the striato-GPe and striatonigral projections. Grade 2 is characterized by 80–95% loss of striatal cannabinoid, D2 dopamine and A2a adenosine receptors, 50% loss of striatal D1 receptors, near complete depletion of D2 and A2a adenosine receptors from GPe, 66% loss of D1 receptors from GPi, 69% loss of D1 receptors from SNr, and greater preservation of cannabinoid receptors in GPi than GPe (Allen *et al.*, 2009; Glass *et al.*, 2000; Richfield and Herkenham, 1994). These findings are consistent with greater preservation of the striato-GPi than the striato-GPe projection at Grade 2, although the finding by Glass *et al.* (2000) of comparable preservation of D1 receptors in GPi and SNr at Grade 2 is inconsistent with greater vulnerability of the latter. At Grade 3, striatum and GPe are nearly devoid of cannabinoid, D2 and A2a receptors, but about 30% of striatal D1 receptors remain, and GPi cannabinoid receptor levels still exceed those in GPe (Allen *et al.*, 2009; Glass *et al.*, 2000; Richfield and Herkenham, 1994). Both GPi and SNr are, however, greatly depleted of D1 receptors by Grade 3, and substantial upregulation of GABAA receptors is evident in GPi (Allen *et al.*, 2009; Glass *et al.*, 2000). These findings too are consistent with greater preservation of the striato-GPi projection than the striato-GPe at Grade 3, but with significant loss of input to GPi. The data of Waeber and Palacios (1989) on 5HT-1 receptors in Grade 3 HD pallidum are also consistent with this conclusion. By Grade 4, these various receptor markers, as well as such intracellular signaling markers as calcineurin, are all greatly reduced in striatum and its targets (Goto *et al.*, 1989b; Glass *et al.*, 2000; Richfield and Herkenham, 1994). This is consistent with near total loss in all striatal projection systems by Grade 4, as well as the neuropathological evidence of severe striatal neuron loss by this grade (Vonsattel *et al.*, 1985).

The attributes that make striato-GPi neurons more resistant than striato-GPe and striatonigral neurons is not known, although considerable attention has focused on the role of glutamate receptor subunit configuration, free radical defenses, calcium sequestering, and anti-apoptotic mechanisms (Beal *et al.*, 1991; Calabresi *et al.*, 1998; Chen *et al.*, 1996, 1998; DiFiglia, 1990; Figueredo-Cardenas *et al.*, 1998; Gervais *et al.*, 2002; Hackham *et al.*, 2000; Hedreen and Folstein, 1995; Huang *et al.*, 1995; Medina *et al.*, 1996; Zeron *et al.*, 2002). Regardless, of their basis, the differential loss explains the progression of HD symptoms (Fig. 9). The early loss of striato-GPe and perhaps striato-SNc neurons accounts for the chorea seen commonly in early HD, according to the now standard direct-indirect pathway model of basal ganglia function (Albin *et al.*, 1989; Crossman, 1987; Deng *et al.*, 2004; Hedreen and Folstein, 1995). Given that each type of striatal projection neuron is organized into microzones that interweave with other types within striatum (Flaherty and Graybiel, 1993; Gimenez-Amaya and Graybiel, 1991),

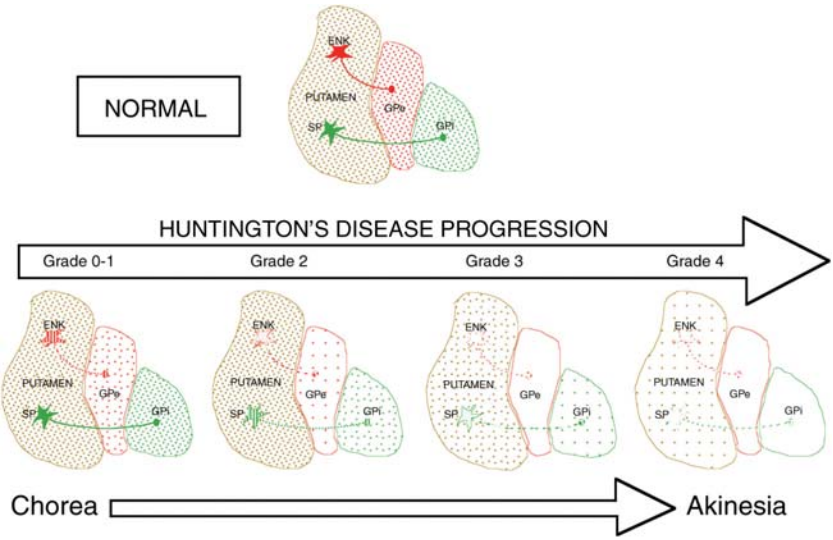


FIG. 9. Schematic illustration of the preferential loss of ENK+ striato-GPe neurons compared to SP+ striato-GPi neurons during the progression of HD, and the relation of this differential loss to HD symptoms. In brief, the early loss of striato-GPe neurons, which suppress unwanted movements, explain the early appearance of chorea in HD, while the later loss of the striato-GPi neurons, which promote desired movement, explain the appearance of akinesia as a later symptom. (For color version of this figure, the reader is referred to the web version of this book.)

the preferential loss of some types of striatal projection neurons in early HD may be why diverse striatal projection neuron markers show patchy loss from striatum (Augood *et al.*, 1996, 1997; Glass *et al.*, 2000; Goto *et al.*, 1989a; Richfield *et al.*, 1991, 1995; Richfield and Herkenham, 1994). Loss of striato-SNr neurons by Grade 1 may cause the saccade abnormalities in early HD since SNr plays a role in saccadic eye movements (Hikosaka, 1989). By Grade 3, considerable loss of striato-GPi neurons appears to occur, and this loss may contribute to the bradykinesia that develops late in HD, while the near complete loss of this projection system by Grade 4 is likely to explain the akinesia in terminal Grade 4 HD (Albin *et al.*, 1989). The functional implications of striato-SNc neuron loss are uncertain, but Tippett *et al.* (2007) indicate that loss of these neurons is associated with mood abnormalities in HD patients. Although differential loss is evident for the four main striatal projection systems, imaging studies assessing brain volume, glucose metabolism, or receptors on striatal projection neurons or their terminals emphasize that neither the striatum itself nor any striatal projection neuron type is completely normal even in premanifest HD (Antonini *et al.*, 1996; Augood *et al.*, 1996, 1997; Aylward *et al.*, 1994,1996; Glass *et al.*, 2000; Grafton *et al.*, 1992; Kuwert *et al.*, 1993; Weeks *et al.*, 1996).

### B. Striatum – Interneurons

Striatal interneurons include (1) very large aspiny cholinergic neurons (Bennett *et al.*, 2000; Kawaguchi *et al.*, 1995); (2) large aspiny neurons that contain GABA and parvalbumin (PARV) (Kawaguchi *et al.*, 1995; Kita *et al.*, 1990); (3) medium-sized aspiny neurons that contain GABA, somatostatin (SS), neuropeptide Y (NPY), and nitric oxide synthase (NOS) (Figueredo-Cardenas *et al.*, 1996a; Kawaguchi *et al.*, 1995); (4) medium-sized aspiny neurons that contain GABA and calretinin (CALR) (Bennett and Bolam, 1993; Cicchetti *et al.*, 2000; Figueredo-Cardenas *et al.*, 1996b; Kawaguchi *et al.*, 1995; Kubota *et al.*, 1993). While the roles of the SS+ and CALR+ interneurons are uncertain, cholinergic and PARV+ interneurons are known to modulate striatal projection neurons (Kawaguchi, 1993; Kawaguchi *et al.*, 1995; Kita *et al.*, 1990; Koos and Tepper, 1999). Cholinergic neurons mediate reward-related (i.e., dopamine-release related) alterations in projection neuron firing, while PARV+ interneurons inhibit striatal projection neurons in a feed-forward manner as part of the process of switching from one movement to the next in a sequence (Berke, 2008; Gage *et al.*, 2010). Cholinergic, SS + , and medium-sized calretinergic striatal interneurons are resistant in HD and survive even late into the disease (Fig. 10) (Albin *et al.*, 1990a; Beal *et al.*, 1986, 1991; Cicchetti and Parent, 1996; Cicchetti *et al.*, 2000; Dawbarn *et al.*, 1985; Ferrante *et al.*, 1985, 1986, 1987a, Ferrante *et al.*, 1987b; Hawker and Lang, 1990; Kowall *et al.*, 1987; Massouh *et al.*, 2008; Norris *et al.*, 1996; Richfield *et al.*, 1995; Sapp *et al.*, 1995). Existing published data, although limited, suggest that PARV+ interneurons may be lost from the striatum as HD progresses (Ferrer *et al.*, 1994; Harrington and Kowall, 1991). This loss may contribute to the worsening motor dysfunction evident as HD progresses. Although SS+ neuron abundance does not decline in HD striatum, expression of NOS and SS in these neurons is progressively diminished (Norris *et al.*, 1996). Similarly, the preservation of cholinergic interneurons in HD striatum is nonetheless accompanied by diminished expression of such cholinergic neuron markers as choline acetyltransferase (Aquilonius *et al.*, 1975; Massouh *et al.*, 2008) and the vesicular acetylcholine transporter (Smith *et al.*, 2006).

### C. Globus Pallidus

In HD, significant progressive atrophy occurs in GPe and GPi, with greater atrophy and gliosis in GPe than GPi (Halliday *et al.*, 1998; Douaud *et al.*, 2006; Roos, 1986; Vonsattel, 2008; Vonsattel and DiFiglia, 1998). The atrophy and gliosis are evident by Grade 3, and prominent by Grade 4 (50%). The shrinkage appears to be due to both neuron loss and loss of striatal input (Lange *et al.*, 1976). Of interest, pallidal shrinkage seems more diagnostic of symptom onset than does striatal

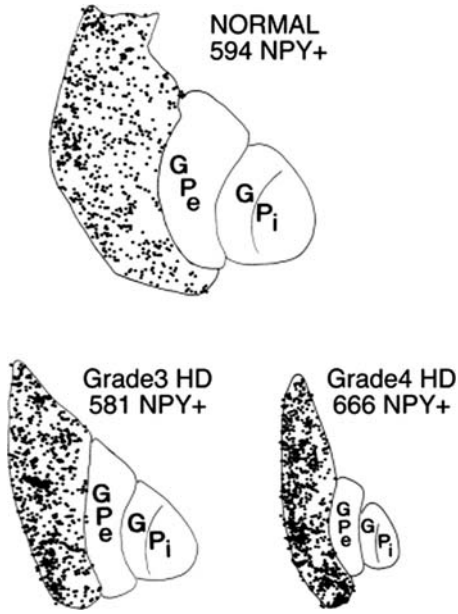


FIG. 10. Camera lucida reconstructions of the distributions of neuropeptide Y-immunoreactive (NPY +) neurons at comparable levels of the basal ganglia, of a normal individual (A), a choreic Grade 3HD case (B), and a rigid Grade 4 HD case (C). Although the number of NPY+ perikarya in putamen is similar, shrinkage of the putamen greatly elevates the packing density of these neurons in Grades 3 and 4 HD. Note also the progressive shrinkage of GPe and GPi in the HD cases. GPe = external globus pallidus; GPi = internal globus pallidus. This illustration is Fig. 2 from Albin *et al.* (1990a).

shrinkage since imaging studies show that striatal shrinkage occurs well before symptoms are manifest, but pallidal shrinkage more immediately precedes symptom appearance (Aylward *et al.*, 1996). The pathophysiological contribution of these pallidal changes is uncertain. In principle, preferential loss of GPe neurons would disinhibit the subthalamic nucleus and contribute to akinesia and possibly rigidity.

## XII. Other Telencephalic Areas in HD

### A. Cerebral Cortex

Cerebral cortex undergoes cell loss, gliosis, and shrinkage in HD, but less so and more slowly than does striatum (Byers *et al.*, 1983; Cudkowicz and Kowall,

1990; De La Monte *et al.*, 1988; Passani *et al.*, 1997; Selemon *et al.*, 2004; Vonsattel *et al.*, 1985). The loss occurs mainly in Layers 3, 5, and 6, is evident over Grades 2–4, and is prominent in Grade 4 (Sotrel *et al.*, 1991). For example, Hedreen *et al.* (1991) noted 57% loss in Layer 6 and 71% loss in Layer 5 in Grade 4 HD. The cortical neuron loss appears to involve the pyramidal projection neurons of cerebral cortex, but not interneurons (MacDonald and Halliday, 2002). For example, neither nNOS nor somatostatin mRNA are significantly decreased in the sensorimotor cortex in HD (Norris *et al.*, 1996), indicating survival of this interneuron class in HD cortex. MRI and fMRI studies show that the cortical thinning is related to disease progress and to CAG repeat length (Kassubek *et al.*, 2004b; Jech *et al.*, 2007), and seems to yield loss of input to striatum (Klöppel *et al.*, 2008; Wolf *et al.*, 2008).

Differences in regional neuron loss and thinning in cerebral cortex occur in HD, and have been described by various authors. Primary motor and premotor cortices both consistently show 40–50% pyramidal neuron loss in late HD (MacDonald and Halliday, 2002). On the other hand, Selemon *et al.* (2004) reported that prefrontal cortex area 9 showed neuron loss but not prefrontal cortex area 46, but both showed shrinkage. Sotrel *et al.* (1993) reported that surviving pyramidal neurons of Layers 3 and 5 in prefrontal cortex showed dendritic augmentation, reflecting perhaps compensation for the loss of other neurons from those layers. MRI and CT imaging studies show results similar to these, revealing that the sensorimotor, insular, and opercular cortices show the most thinning, while frontal and temporal cortices show relatively less (Douaud *et al.*, 2006; Kassubek *et al.*, 2004b; Mühlau *et al.*, 2007; Rosas *et al.*, 2003), with thinning in these areas manifest even before overt HD motor symptoms and associated with decline in cognitive function as measured by the UHDRS (Rosas *et al.*, 2005). Heterogeneity in HD in motor versus mood symptoms appears in part attributable to regional variation in cortical neuron loss since a significant association between motor dysfunction and neuronal loss in primary motor cortex is seen in HD, as well as between mood disturbance and neuronal loss in anterior cingulate cortex (Thu *et al.*, 2010). Braak and Braak (1992) reported loss of entorhinal cortex neurons in advanced HD, suggesting a basis for memory deficits in late HD.

Functional alterations in neurotransmitter release also occur for neurons of cerebral cortex, and may underlie HD symptoms. For example, a loss of various presynaptic proteins, such as the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein, synaptosome-associated protein 25 (SNAP 25), and the vesicle docking and recycling protein rabphilin 3a, occurs in frontal cortex in HD Grades 1–4 (Morton *et al.*, 2001; Smith *et al.*, 2007). These losses are not due to a general loss of synapses in HD cortex (Smith *et al.*, 2007). Similarly, Zucker *et al.* (2010) showed that Layer 5 motor cortex neurons in HD make less Lin7 homolog b (Lin7b, also known as veli-2 and mals2), which is a scaffold protein implicated in synaptic plasticity and neurite outgrowth. These

types of changes could impair synaptic function within cortex and between cortex and striatum. In HD, uptake of glutamate was found to be reduced by 43% in prefrontal cortex, with the defect increasing in severity with CAG repeat expansion; impairment of glutamate uptake may contribute to neuronal dysfunction and pathogenesis in HD (Hassel *et al.*, 2008).

### B. Amygdala

The amygdala comprises pallial and subpallial subdivisions. Significant amygdala shrinkage has been reported in HD, based on MRI and CT (Douaud *et al.*, 2006; Rosas *et al.*, 2003), and Kipps *et al.* (2007) reported declining emotion recognition in others with amygdala volume loss in HD, possibly contributing to HD affective symptoms. Zech *et al.* (1986) reported that the central nucleus of the subpallial amygdala in one choreiform HD case was markedly shrunken, with considerable attenuation of immunolabeling for VIP, ENK, neurotensin, and NPY.

## XIII. Brainstem Areas in HD

### A. Thalamus

Thalamus and the subthalamic nucleus undergo shrinkage and cell loss in HD (Byers *et al.*, 1973; Douaud *et al.*, 2006; Mann *et al.*, 1993; Vonsattel *et al.*, 1985). The centre median, for example, shows evident neuronal loss and astrogliosis by Grade 3 (Vonsattel, 2008), and together the centromedian/parafascicular nucleus complex shows about a 25% volume loss and 50% neuron loss in advanced HD (Heinsen *et al.*, 1996), while only 15% neuron and volume loss is seen in mediodorsal nucleus (Heinsen *et al.*, 1999). Up to 25% volume loss is observed in subthalamic nucleus by Grade 4 (Lange *et al.*, 1976). It is uncertain if this reflects neuron loss or loss of GPe input. Ventrobasal thalamus also shows atrophy (Dom *et al.*, 1976). Imaging studies indicate that thalamic nuclei projecting to frontal cortex and/or striatum (dorsomedial, centre median, parafascicular, and ventrobasal) undergo considerable atrophy in HD, and their atrophy is associated with affective symptoms (Kassubek *et al.*, 2004a).

### B. Hypothalamus

Many of the nonmotor symptoms of HD, such as weight loss, sleep abnormalities, hypometabolism, and muscle atrophy are unexplained. Given the central role

of hypothalamus in these functions, attention has recently focused on the impact of HD on hypothalamus. Studies using voxel-based morphometry of MR images or CT have shown hypothalamic atrophy in early HD patients (Douaud *et al.*, 2006; Kassubek *et al.*, 2004a,b), and significant hypothalamic atrophy (as reflected in ventricular expansion) is evident even 10 years before estimated symptom onset (Soneson *et al.*, 2010). Among specific nuclei, atrophy of the lateral tuberal nucleus (Kremer *et al.*, 1990), reflecting loss of somatostatinergic neurons (Timmers *et al.*, 1996), has been seen in HD. Notably with regard to sleep disorders in HD, a 28% loss and a 27% atrophy of neurons expressing the neuropeptide orexin has been noted in the lateral hypothalamic area of HD patients (Petersén *et al.*, 2005). The lateral hypothalamus contains neuronal populations important for regulation of sleep and wakefulness, and feeding (DiLeone *et al.*, 2003). As loss of orexinergic neurons is associated with narcolepsy and obesity (Kok *et al.*, 2003), their loss in HD is unlikely to be involved in HD-related weight loss but may be contributory to the sleep defects. Given that the somatostatin and orexin cell populations are small, and atrophy of the hypothalamus is prominent in HD, diverse hypothalamic populations are likely to be affected in HD. Since oxytocin and vasopressin neurons were decreased by 45% and 24%, respectively, in advanced HD, it seems likely that paraventricular and supraoptic nucleus loss contributes to HD hypothalamic shrinkage (Gabery *et al.*, 2010). The numbers of NPY neurons (many of which are involved in feeding suppression) is, however, unchanged (Gabery *et al.*, 2010). Detailed characterization of hypothalamic neuropathology in HD, its progression, and its relation to the nonmotor symptoms of HD is still, however, limited.

### C. Substantia Nigra

Substantia nigra undergoes cell loss and shrinkage in HD, but less so than does striatum (Byers *et al.*, 1983; Cudkowicz and Kowall, 1990; De La Monte *et al.*, 1988; Sharp and Ross, 1996; Vonsattel *et al.*, 1985). Shrinkage of substantia nigra as detected by CT has also been reported, which could stem from both striatal input loss and nigral neuron death (Douaud *et al.*, 2006). Loss of both SNr GABAergic neurons, and SNc dopaminergic neurons is evident in HD (Vonsattel, 2008), and Oyanagi *et al.* (1989) have reported 40% loss in both populations. Yohrling *et al.* (2003) reported that in Grade 4 HD tyrosine hydroxylase expression by dopaminergic neurons, as detected by *in situ* hybridization, was decreased by 46% per surviving dopaminergic neuron. Moreover, the dopaminergic neurons were 33% smaller than normal. Neuron counts were not, however, performed to assess dopaminergic neuron loss in that study. Consistent with the reduced tyrosine hydroxylase expression, Yohrling *et al.* (2003) additionally found that tyrosine hydroxylase protein in the nigra was reduced by 32%. They

attributed the reduced tyrosine hydroxylase expression to an effect of mutant huntingtin on the tyrosine hydroxylase promoter. As would be predicted from loss of nigral dopaminergic neurons and reduced tyrosine hydroxylase expression, terminals containing tyrosine hydroxylase appear to be reduced in abundance in advanced HD striatum (Ferrante and Kowall, 1987). Dopamine and its metabolite HVA, and VMAT2 have also been reported to be reduced in HD striatum by some authors (Bohnen *et al.*, 1986; Bohnen *et al.*, 2000; Kish *et al.*, 1987; Reynolds and Garrett, 1986). Loss of dopamine input could contribute to akinesia in HD, as it does in Parkinson's disease.

### D. Cerebellum

Volumetric loss and sporadic Purkinje cell loss is evident in HD Grades 3 and 4, notably in juvenile onset victims (Castaigne *et al.*, 1976; Hattori *et al.*, 1984; Jeste *et al.*, 1984; Rodda, 1981; Vonsattel, 2008). Amino acid and neuropeptide neurotransmitter levels, and GABA receptor levels appear to be largely normal in HD cerebellum (Beal *et al.*, 1988; Kish *et al.*, 1983).

### E. Brainstem

Significant loss of neurons from diverse brainstem regions and overall brainstem shrinkage has also been reported in HD (Hattori *et al.*, 1984). For example, Koeppen (1989) reported about 30% loss from the midline pontine region controlling saccades, and linked this loss to saccadic defects in HD.

## XIV. HD and Neurogenesis

In response to striatal injury, the subventricular zone (SVZ) of the caudate increases the production of progenitor cells that migrate toward the site of the injury where they can differentiate into mature neurons and glia as part of a restorative process (Curtis *et al.*, 2007). Curtis *et al.* (2003) showed an increase in cell proliferation in the SVZ in HD caudate, progressive with HD grade and CAG repeat, using the cell cycle marker proliferating cell nuclear antigen (PCNA). Proliferating cells were shown to express the neuronal marker beta III-tubulin or the glial cell marker GFAP, demonstrating generation of neurons and glial cells in the SVZ of HD caudate. The SVZ of HD caudate is 2.8-fold thicker than normal at Grade 2/3, with thickness increasing with grade. An increase in glial

cells is mainly responsible for the large increase, but neuroblasts and progenitor cells are also increased in abundance (Curtis *et al.*, 2005a,b).

## XV. Neuroinflammatory Neuropathology in HD

Microglial activation and the associated neuroinflammation appear to be a prominent pathological feature of HD, evident from early in the disease process (Tai *et al.*, 2007). For example, activated microglia are greatly increased in abundance in HD cortex, striatum, and globus pallidus, and their abundance increases with grade and neuron loss (Pavese *et al.*, 2006; Sapp *et al.*, 2001a). Similarly, the expression of the neuroinflammation mediators, CCL2 and IL-10, is increased specifically in the striatum in HD, presumably in activated microglia, but not in cortex or cerebellum (Silvestroni *et al.*, 2009). By contrast, an upregulation of the neuroinflammation mediators IL-6, IL-8, and MMP9 is seen in cortex and the cerebellum. The activated microglia may be neurotrophic and act to combat the HD pathogenic process, or their sustained activation may exacerbate the HD injury process (Möller, 2010).

## Acknowledgments

Our research on Huntington's disease has been supported by Cure HD Contracts from the Hereditary Disease and High Q Foundations (AR/ID), the Hereditary Disease Foundation (AR), and NIH grants NS19620 (AR), NS28721 (AR).

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# PATHOGENIC MECHANISMS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder presenting in midlife. Multiple pathogenic mechanisms which hypothesise how the expanded CAG repeat causes manifest disease have been suggested since the mutation was first detected. These mechanisms include events that operate at both the gene and protein levels. It has been proposed that somatic instability of the CAG repeat could underlie the striatal-specific pathology observed in HD, although how this occurs and what consequences this has in the disease state remain unknown. The form in which the Htt protein exists within the cell has been extensively studied in terms of both its role in aggregate formation and its cellular processing. Protein-protein interactions, post-translational modifications and protein cleavage have all been suggested to contribute to HD pathogenesis. The potential downstream effects of the mutant Htt protein are also noted here. In particular, the adverse effect of the mutant Htt protein on cellular protein degradation, subcellular transport and transcription are explored, and its role in energy metabolism and excitotoxicity investigated. Elucidating the mechanisms at work in HD pathogenesis and determining when they occur in relation to disease is an important step in the pathway to therapeutic interventions.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00015-8




0074-7742/10 $35.00

## I. Introduction

Multiple pathogenic mechanisms have been suggested to underlie Huntington's disease (HD). Some were postulated before the gene was cloned in 1993 (Huntington's Disease Collaborative Research Group, 1993) and many more have been suggested since (Bates *et al.*, 2002; Shao and Diamond, 2007). There is substantial evidence that many of these mechanisms occur as part of the disease or are seen in disease models. As yet, however, there is no complete picture of the molecular events and pathogenic mechanisms that mediate how the expanded CAG repeat in the *HTT* gene manifests the complex symptoms of the disease. It is entirely possible that multiple mechanisms are involved in initiating and propagating HD. Elucidating the mechanisms central in disease manifestation and progression is important in enabling targetted clinical trials of HD treatments to take place.

HD is one of a series of diseases caused by expanded CAG repeats in a gene that are translated to glutamine in the encoded proteins. All are neurodegenerations and their different signs and symptoms are most likely the result of the context of the protein in which the expanded glutamine tract resides and the tissue and cellular expression pattern of the genes (Orr and Zoghbi, 2007; Shao and Diamond, 2007; Truant *et al.*, 2007). The mechanisms that might underlie HD discussed here include the role of somatic instability and *HTT* RNA in HD, events that operate on the huntingtin (Htt) protein itself and events that are downstream of the Htt protein including transcriptional dysregulation, transport defects, energy metabolism, and mitochondrial dysfunction and excitotoxicity.

## II. The *HTT* Gene Product

The huntingtin mRNA and protein produced from the gene give rise to the disease symptoms of HD. The RNA is very widely expressed and has been found in most tissues examined (Huntington's Disease Collaborative Research Group, 1993). Two major mRNA species are produced with different 3′ UTRs but the same protein product encoded (Huntington's Disease Collaborative Research Group, 1993). It is possible that the expansion carrying RNA contributes to the disease, by a mechanism similar to that operating in myotonic dystrophy (DM) where the CUG-containing RNA is retained in the nucleus and alters alternative splicing (Orr and Zoghbi, 2007; Todd and Paulson, 2010). This is made more likely by the observation of somatic expansion of the *HTT* CAG repeat in many cell types, especially those most affected in the disease. Somatic expansion of the CAG repeat may also lead to longer repeats being present in susceptible cells than are seen in the blood, with concommitantly more severe toxic effects in those cells.

If there is no gene product there is no disease: perhaps the most important experimental evidence to indicate that HD may be amenable to therapy is that

produced by silencing the mutant gene. Yamamoto *et al.* (2000) used a tetracyline inducible system to switch off the expression of a 90Q exon1 mutantHtt (mHtt) fragment in mice. When the gene was switched off the mice recovered behaviorally and the mHtt-positive inclusions that could be observed in the brain before the gene was switched off disappeared. This implies that provided neurons are still present in the brain, removing the toxic insult of mHtt allows them to recover functionally. More recent evidence using RNA-silencing methods is consistent with this result (Boudreau *et al.*, 2009; DiFiglia *et al.*, 2007; Drouet *et al.*, 2009; Harper *et al.*, 2005; Pfister *et al.*, 2009). Such silencing therapies, which prevent the mutant gene from expressing its toxic effects, if successful, may well preclude the need to understand all of the functional consequences of the mutation. They also imply that at risk HD gene carriers would ideally need to be treated before the onset of the disease at which point substantial brain atrophy has occurred and neurons have been lost (Tabrizi *et al.*, 2011; Vonsattel *et al.*, 1985). However, it is not clear that adequate gene silencing can be achieved either technically nor what the physiological side-effects of such silencing might be (Davidson and Boudreau, 2007; Harper, 2009; Pfister and Zamore, 2009). Reduction of normal Htt expression in mice to below 50% gave rise to severe neurological abnormalities (Auerbach *et al.*, 2001). Any silencing therapy is likely to take some time to come into routine clinical use, may not deal with all of the symptoms and in addition there are always likely to be people who manifest the disease without knowing they are at risk, the so-called new mutations (Falush *et al.*, 2001; Semaka *et al.*, 2010), thus understanding the pathogenic mechanisms in HD is essential.

### A. Somatic Expansion of the *HTT* Gene

The instability of the CAG repeat in *HTT* was noted when the gene was first cloned (Huntington's Disease Collaborative Research Group, 1993) and this observation has been confirmed both in the germline (Telenius *et al.*, 1994) and in somatic cells (Aronin *et al.*, 1995; Cannella *et al.*, 2009; Gonitel *et al.*, 2008; Kahlem and Djian, 2000; Swami *et al.*, 2009; Veitch *et al.*, 2007) in HD patients and also in mouse models of the disease (Gonitel *et al.*, 2008; Ishiguro *et al.*, 2001; Lloret *et al.*, 2006; Kennedy *et al.*, 2003; Kennedy and Shelbourne, 2000; Shelbourne *et al.*, 2007; Vatsavayai *et al.*, 2006). The inverse correlation of the CAG repeat length, usually measured in blood, with the age of onset of HD (Langbehn *et al.*, 2004, 2010) demonstrates that increased length of the CAG repeat and its translated glutamine tract are central to disease pathogenesis so somatic expansion could be important in disease onset and progression.

How does somatic expansion of repeats in the DNA of terminally differentiated cells occur? DNA undergoes a number of processes that involve cutting, unwinding, and replication of sections of DNA, including transcription and repair. The mechanisms underlying the expansion of the CAG repeat in somatic cells appear

to be mediated by DNA repair and in dividing cells also by replication (see McMurray, 2010 for a detailed explanation). Both base-excision repair (BER) and mismatch repair (MMR) have been implicated in *in vivo* somatic expansion in mouse model brains (Dragileva *et al.*, 2009; Kovtun *et al.*, 2007; Kovtun and McMurray, 2001; Manley *et al.*, 1999; Wheeler *et al.*, 2003).

The most extreme somatic expansions of *HTT* occur in the striatum in both human HD brain and mouse model brain (Gonitel *et al.*, 2008; Goula *et al.*, 2009). Greater somatic expansion of the repeat in the cortex of HD subjects was associated with earlier disease onset (Swami *et al.*, 2009) indicating that factors contributing to somatic expansion are likely to be important in disease manifestation. Kovtun *et al.* (2007) showed that the somatic expansion seen in R6/1 mice was at least partly dependent on the base excision repair enzyme 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1). This enzyme is involved in the repair of DNA damaged by oxidative processes and removes lesions and in the mice such damage accumulated as the mice aged and correlated well with the increase in somatic expansion of the CAG repeat in the *Htt* gene. As all tissues show oxidative DNA damage, Goula *et al.* (2009) showed that the tissue specificity of the expansions might be a result of the relative expression of Flap endonuclease-1 (FEN1) which is involved in Okazaki fragment processing during base excision repair of DNA. The mouse mismatch repair genes *Msh2* and *Msh3* both contributed to somatic expansion in the HD knock in mouse line HdhQ111 (Wheeler *et al.*, 2003) and *Msh6* contributed to germline changes in CAG length.

HD is not the only repeat expansion disease and the context of the repeats in which the expansions lie is likely to be important. It seems that processes that operate on DNA mediate the expansion of the CAG repeat in both germline and somatic cells. These are likely to be different in different cell types but the underlying mechanisms are not yet clear. However, the evidence suggests that these expansions may be an important contributor to disease manifestations. It is not clear whether one mechanism predominates in specific cells or whether multiple mechanisms can occur. The different lengths of expansion could well be mediated in different ways. It is possible that some of the proteins implicated in repair might be involved in multiple pathways (McMurray, 2010). It is also important to elucidate whether the expanded repeats in cells are transcribed to RNA, and whether the expansion affects the efficiency of this process and whether the RNA is subsequently processed normally and translated into protein with somatically expanded polyglutamine repeat lengths. There is evidence in juvenile patients for expanded mHtt consistent with somatically expanded Htt mRNA being translated into protein (Aronin *et al.*, 1995).

### B. The Htt Protein and its Processing

The full native folded form of either mutant or wild-type Htt protein remains unknown, but studies have revealed some clues to its three-dimensional structure and

these may inform our understanding of its functions. It is known to be a stable protein with a relatively long half-life probably in excess of 24 h (Persichetti *et al.*, 1996). Compared with the wild-type protein, mHtt fragments have a longer half life and are more susceptible to accumulating in the cell (Kaytor *et al.*, 2004). The N-terminus of Htt is thought to have an external loop structure (Atwal *et al.*, 2007; Li *et al.*, 2006). There are two consecutive polyproline repeat regions immediately following the polyglutamine repeat, a motif often found in transcriptionally active proteins (Huntington's Disease Collaborative Research Group, 1993). Much of the rest of the protein appears to contain a series of four HH-HEAT repeats (Li *et al.*, 2006; Takano and Gusella, 2002; Xia *et al.*, 2003): this motif is thought to be important in mediating protein–protein interactions (Neuwald and Hirano, 2000). In addition, there are proteolytically susceptible PEST domains (Schilling *et al.*, 2006).

Htt is observed in the nucleus and the cytoplasm and it has been suggested that it functions as a nucleocytoplasmic shuttle protein, like many of the proteins that carry glutamine tracts and cause polyglutamine diseases (Truant *et al.*, 2007). A nuclear export signal has been demonstrated toward the C-terminus of the protein (Xia *et al.*, 2003) though no nuclear localization signal has been detected. There has long been a debate about the localization of Htt and its cleavage products but it is now clear that both Htt and mHtt proteins can be observed in the cytoplasm and nucleus of cells (DiFiglia *et al.*, 1997; Kegel *et al.*, 2002). N-terminal fragments of normal and mutant Htt appear to be at higher concentration in the nucleus than the full length protein: this may be due to the NES being relatively C-terminal and inhibiting nuclear export (Truant *et al.*, 2007). Benn *et al.* (2005) demonstrated that directing exon 1 of human mHtt to the nucleus generated a more severe and rapid phenotype than directing it to the cytoplasm, but that even the extranuclear forms of the mutant protein caused neurodegeneration and features such as dystrophic neurites characteristic of HD.

Many protein interactions with the N-terminus of both Htt and mHtt have been published but very few have been detected with the more C-terminal regions of Htt. This may be because there are fewer such interactions, but Htt is a large protein and many of the systems used to detect such interactions would require sections of Htt to be used and as its three dimensional structure is unknown, this renders the use of physiologically relevant fragments difficult. In addition, Htt is known to be cleaved (see section, II E) and thus many studies have only used various N-terminally truncated fragments to look for interacting proteins.

### C. Htt Aggregation and Inclusions

It has been well established that the mutant Htt protein containing an expanded polyglutamine repeat has the propensity to misfold and form aggregates (Davies *et al.*, 1997). This is also a property of other polyglutamine-repeat containing proteins (reviewed in Orr and Zoghbi, 2007).

Evidence of inclusions was initially presented in the 1970s (Roizin *et al.*, 1979) and studied in more detail following the development of the first HD mouse models which contained human exon 1 of *HTT* with around 115 (R6/1) or 150 (R6/2) CAG repeats (Mangiarini *et al.*, 1996). The immunohistochemical study of the HD R6 transgenic mouse lines demonstrated the presence of a single nuclear inclusion (NII) in most striatal neurons, which were human Htt and ubiquitin-positive, yet devoid of the normal endogenous mouse Htt. Inclusions were also noted in neurons of cerebral cortex, cerebellum, and spinal cord (Davies *et al.*, 1997). Table I summarizes neuropathological inclusion findings in multiple mouse models of HD.

These findings were swiftly followed by the observation of similar mHtt containing structures in human post-mortem brain tissue from HD patients (Becher *et al.*, 1998; DiFiglia *et al.*, 1997; Gourfinkel-An *et al.*, 1998; Gutekunst *et al.*, 1999; Maat-Schieman *et al.*, 1999). The frequency (Becher *et al.*, 1998) and rate of formation of such aggregates (Scherzinger *et al.*, 1999) were shown to be polyglutamine repeat length dependent (Lakhani *et al.*, 2010). Aggregate size also appears to increase with duration of disease (Gutekunst *et al.*, 1999). In the brains of juvenile and adult-onset HD cases, nuclear inclusions were detected in the cortex and striatum but were absent from the globus pallidus and cerebellum (DiFiglia *et al.*, 1997). In addition to NIIs, inclusions were also demonstrated in dystrophic neurites of the striatum and cortex (DiFiglia *et al.*, 1997). The inclusions were shown to be composed primarily of N-terminal mHtt fragments (DiFiglia *et al.*, 1997; Hackam *et al.*, 1998; Maat-Schieman *et al.*, 1999; Martindale *et al.*, 1998).

Since these initial findings mHtt aggregates have been observed in many other animal models including *Drosophila melanogaster* (Warrick *et al.*, 1998) and *C. elegans* (Faber *et al.*, 1999; Parker *et al.*, 2001). Many HD cellular models have also demonstrated mHtt-positive inclusions (Carmichael *et al.*, 2000; Ho *et al.*, 2001; Lunkes and Mandel, 1998; Ratovitski *et al.*, 2007; Saudou *et al.*, 1998).

MHtt inclusions have been observed not only in CNS but in peripheral tissues in mouse models and in people (Tabrizi *et al.*, 2000). In both the R6/2 mice (Sathasivam *et al.*, 1999) and the Q150 mice (Moffitt *et al.*, 2009) inclusions were detected in a wide range of tissues including skeletal muscle, heart, liver, adrenal glands, pancreas, kidney, stomach wall, and duodenum at late stages of phenotype development.

Structurally, inclusions appeared as a mixture of granules, filaments, and fibrils and were not separated from their surroundings by a membrane (DiFiglia *et al.*, 1997). GST-fusion proteins of mHtt exon 1 were cleaved and subsequently formed protein aggregates that resembled amyloid with a $\beta$-pleated sheet structure (Scherzinger *et al.*, 1997). This was consistent with the $\beta$-pleated sheet structure formed by poly-L-glutamines which indicated that expanded polyglutamine may act as polar zippers (Perutz, 1996; Perutz *et al.*, 1994). The mHtt fibrils have been shown to have extensive branched morphologic features which become more advanced at the later stages of degeneration (Dahlgren *et al.*, 2005).

Table I
NEUROPATHOLOGY IN HUNTINGTON'S DISEASE MOUSE MODELS

| Mouse Model | Nature of Model | Location of Neuronal Inclusions | Reference |
|---|---|---|---|
| R6/1, R6/2, R6/5 (Q115, 150) | Transgenic, human exon 1 Htt | striatum, cortex, cerebellum, spinal cord | Mangiarini *et al.*, 1996 Davies *et al.*, 1997 |
| N171-82Q | Transgenic, human truncated Htt | striatum, cortex, hippocampus, cerebellum | Schilling *et al.*, 1999 |
| YAC72 | Transgenic, human FL Htt | striatum | Hodgson *et al.*, 1999 |
| HD48, HD89 | Transgenic, human FL Htt | striatum, cortex, hippocampus, thalamus, cerebellum | Reddy *et al.*, 1998 |
| BACHD (97Q) | Transgenic, human FL Htt 97Q | cortex and striatum | Gray *et al.*, 2008 |
| HD46, HD100 | Transgenic, human truncated Htt | striatum and cortex | Laforet *et al.*, 2001 |
| $Hdh^{Q92}$, $Hdh^{Q111}$ | Knock-in | striatum, cortex, olfactory tubercle/bulb, nucleus accumbens, cerebellum, hippocampus, septum | Wheeler *et al.*, 2000 |
| HD knock-in mice (72-80 CAG) | Knock-in | striatum | Li *et al.*, 2001 Shelbourne *et al.*, 1999 |
| $Hdh^{(CAG)150}$ | Knock-in | striatum, cortex, nucleus accumbens, hippocampus, cerebellum | Lin *et al.*, 2001 |
| HD94 | Knock-in | striatum | Menalled *et al.*, 2002 |
| HD140 | Knock-in | striatum, nucleus accumbens and olfactory tubercle | Menalled *et al.*, 2003 |
| HdhQ200 | Knock-in | striatum and cortex | Heng *et al.*, 2010 |
| HD[CAG]94 (conditional) | Transgenic, human exon 1 Htt, inducible | striatum and cortex | Yamamoto *et al.*, 2000 |
| PrP-tTA-6/iFL23Q-1 and 148Q-69 | Transgenic, human FL Htt 148Q, inducible | cortex, striatum, hippocampus, cerebellum | Tanaka *et al.*, 2006 |

Numbers of CAG repeats or glutamines (Q) in translated mutant protein given if not contained in mouse name. FL = full length.

Intranuclear and extranuclear inclusions are structurally different with extranuclear inclusions appearing more diverse in shape and size in R6/2 mice (Morton *et al.*, 2000). Formation of NIIs was followed by nuclear membrane invagination and an increase in the density of nuclear pores (Davies *et al.*, 1999) and inclusions in dystrophic neurites formed after NIIs in R6/2 mice. Lipofuscin

then accumulates in the cytoplasm (Davies *et al.*, 1997; Vonsattel and DiFiglia, 1998) and neurodegeneration results (Turmaine *et al.*, 2000).

The dynamics of inclusion formation have been studied in an attempt to elucidate how the protein transforms from a monomeric state into oligomeric compounds and then aggregates (Colby *et al.*, 2006; Olshina *et al.*, 2010; Ramdzan *et al.*, 2010; Sathasivam *et al.*, 2010; Thakur *et al.*, 2009). The identity of the toxic species in HD and its link with pathogenesis remain to be defined and soluble oligomers rather than frank aggregates have been suggested to be the toxic moiety (Caughey and Lansbury, 2003). The possible relationship of the aggregating protein to toxicity is outlined in Fig. 1 and evidence supporting their role in HD pathogenesis is summarized in Table II. Aggregates could be harmful in HD as they sequester proteins such as those involved in cellular transport, transcription factors, UPS components, and wild-type Htt (all discussed below). By contrast, aggregates could be protective through the sequestration of the putative soluble toxic Htt moiety or other cellular proteins, for instance mTOR, which stimulate mutant Htt clearance (Ravikumar *et al.*, 2004). However, as Htt inclusions are exclusive to HD tissues, it is reasonable to study their involvement in the pathogenesis of HD.

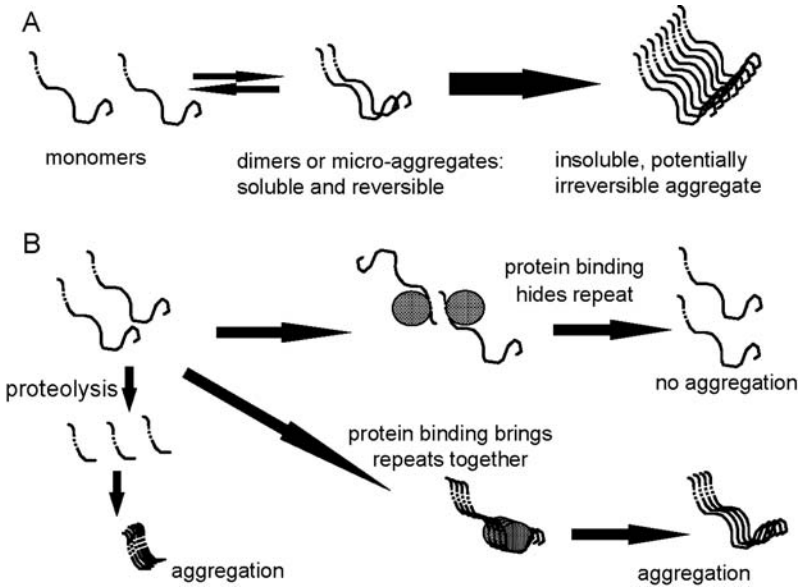


FIG. 1. The potential dynamics of mHtt aggregation. (A) shows the potential aggregation pathway of mHtt. The monomer binds into aggregates in a reversible fashion. Once aggregation starts to occur this will drive the entire reaction to the right, and remove monomers from solution. Many parameters are likely to influence this including the absolute concentration of mHtt: there is likely to be a mass action effect. (B) shows events that might influence the propensity of this reaction to cause aggregates including cleavage of mHtt and the binding of interacting proteins.

Table II
HUNTINGTIN AGGREGATES AND HD PATHOGENESIS

| | |
|---|---|
| **Huntingtin Inclusions are Harmful** | |
| Inclusion formation coincides with behavioral changes in R6/2 mice | Morton *et al.*, 2000 |
| Switching off mHtt in an inducible HD mouse model results in aggregate clearance and reversal of phenotype | Yamamoto *et al.*, 2000 |
| Chaperone activity can decrease the toxicity induced by expanded polyglutamine | Chai *et al.*, 1999 |
| Glial cells have no inclusions and do not degenerate in HD | Gourfinkel-An *et al.*, 1998 |
| Compounds capable of interfering with aggregate formation can rescue toxicity in HD | Colby *et al.*, 2004<br>Ehrnhoefer *et al.*, 2006 |
| Nuclear and cytoplasmic inclusions form prior to the onset of cell toxicity in an inducible PC12 HD cell model | Ratovitski *et al.*, 2007 |
| Bacterial and yeast chaperones can reduce aggregate formation and cell death in mammalian models of HD | Carmichael *et al.*, 2000 |
| Inclusion formation is correlated with enhanced apoptosis | Lunkes and Mandel, 1998 |
| Polyglutamine aggregates introduced into cells in culture cause cell death | Yang *et al.*, 2002 |
| **Huntingtin Inclusions are Incidental** | |
| Inclusions are less common in the striatum, where most neurodegeneration occurs in HD, than in the cortex | Gutekunst *et al.*, 1999 |
| Htt inclusions detected after the initial onset of motor and cognitive dysfunction and neuronal loss | Menalled *et al.*, 2003<br>Slow *et al.*, 2003 |
| Neurodegeneration can occur in the absence of aggregates | Hodgson *et al.*, 1999 |
| Inclusions form in the absence of cell death | Wheeler *et al.*, 2000 |
| Caspase inhibition reduced inclusions but did not increase cell survival | Kim *et al.*, 1999 |
| RNA levels were reduced in R6/2 striatal neurons with and without inclusions | Sadri-Vakili *et al.*, 2006 |
| **Huntingtin Inclusions are Protective** | |
| R6/2 crossed with tissue transglutaminase knock-out mice rescued brain and body weight loss and early mortality but increased inclusions | Mastroberardino *et al.*, 2002 |
| Neurons containing htt inclusions had reduced mutant htt levels elsewhere in the neuron and improved survival | Arrasate *et al.*, 2004 |
| Htt inclusions sequester mTOR which promotes autophagy and enhances mt htt clearance from the cell | Ravikumar *et al.*, 2004 |
| Inclusions more common in striatal interneurons spared in HD compared with spiny neurons that degenerate | Kuemmerle *et al.*, 1999 |
| Suppressing inclusion formation enhanced cell death | Saudou *et al.*, 1998 |
| Aggregates found in brain regions spared in HD | Reddy *et al.*, 1998 |
| An inverse correlation was shown between aggregate formation and cell toxicity | Kaytor *et al.*, 2004 |
| Reducing inclusion formation increased cell death of neurons expressing mutant htt | Okamoto *et al.*, 2009 |

### D. Post-translational Modification

Htt is subject to a number of post-translational modifications, some of which are mediated by glutamine length and have been associated with pathogenesis. Post-translational modifications involve the addition of other molecular moieties to proteins which can often change their localization and function (Appella and Anderson, 2001; Hunter, 2007). Sumoylation, acetylation, palmitoylation, phosphorylation, and ubiquitination have all been demonstrated to occur at different points along Htt and to alter its localization and function.

Htt is phosphorylated at multiple sites (Aiken *et al.*, 2009; Humbert *et al.*, 2002; Schilling *et al.*, 2006; Thompson *et al.*, 2009) and modulation of phosphorylation can alter the toxicity of mHtt (Aiken *et al.*, 2009; Gu *et al.*, 2009; Humbert *et al.*, 2002; Luo *et al.*, 2005; Metzler *et al.*, 2010; Rangone *et al.*, 2004; Thompson *et al.*, 2009). Humbert *et al.* (2002) detected an Akt-mediated phosphorylation of Htt at Ser421 (S421) that ameliorated neurotoxicity of mHtt in cultured cells, but did not appear to affect cleavage. Intracellular transport is affected by reduced phosphorylation of S421 in mHtt (Colin *et al.*, 2008; Gauthier *et al.*, 2004) potentially reducing trophic support, and phosphorylation of S421 is reduced by NMDA stimulation in YAC128 primary neurones (Metzler *et al.*, 2007, 2010). This dephosphorylation is mediated by the protein phosphatases PP1 and PP2A. Conversely, increased phosphorylation of S421 is protective to cells (Pardo *et al.*, 2006). Again in cell cultures transfected with full length huntingtin with normal (23) and expanded (148) glutamine tracts, Htt was shown to be constitutively phosphorylated at a number of other more C-terminal Ser residues, of which three were ERK1 mediated, rendered S533 incapable of phosphorylation and reduced mHtt toxicity, most likely by reducing calpain cleavage of the protein (Schilling *et al.*, 2006).

The N-terminal phosphorylations of Htt, in the 17 amino acids before the glutamine tract, have also been studied. S13 and S16 were mutated in BAC-HD mutant mice to mimic constitutive phosphorylation at both residues and this ameliorated the HD phenotype of these mice (Gu *et al.*, 2009). The S13 phosphorylation was mediated by IKK$\alpha$ and $\beta$ though it was not clear how the S16 phosphorylation was modulated (Thompson *et al.*, 2009). These N-terminal phosphorylations appear to target Htt for degradation (Thompson *et al.*, 2009). T3 phosphorylation, the most frequent N-terminal modification observed, also alters toxicity of the exon 1 fragment of mHtt: mutations which mimic constitutive phosphorylation (T3D) and which are incapable of phosphorylation (T3A) both reduce cell death (Aiken *et al.*, 2009). The mechanisms of protection are likely to be different though as T3D increases and T3A reduces aggregation of mHtt. Exon 1 Htt protein carrying the expanded glutamine was less heavily phosphorylated than the wild-type exon 1 protein. So Htt is phosphorylated at multiple sites and the phosphorylations are important in the fate of the mutant protein. It remains unclear exactly how the expanded glutamine tract affects all of the

phosphorylations and which phosphorylations occur early in the modification program of Htt. However, phosphorylation of mHtt generally seems to protect cells against the toxic effects of the protein.

The phosphorylations of Htt also seem to promote other modifications including ubiqutination, SUMOylation, and acetylation of the N-terminal lysines (Thompson *et al.*, 2009). Both K6 and K9 could be SUMOylated and ubiquitinated. SUMOylation reduced the ability of N-terminal Htt to form aggregates in cell culture and promoted transcriptional repression (Steffan *et al.*, 2004) but in the Htt exon 1 *Drosophila* model of HD, SUMOylation exacerbated neurodegeneration whereas ubiquitination proved protective. The N-terminus of Htt is acetylated at K9 and there is a further acetylation at K444: the K444 acetylation mediates trafficking of Htt to autophagosomes and mutant Htt mutated at K444 to prevent acetylation leads to accumulation and neurodegeneration both in cultured neurons and mouse brain (Jeong *et al.*, 2009). It is possible that histone deacetylase inhibitors exert their protective effects by directly increasing mHtt acetylation and degradation.

Htt is also modified by the fatty acid palmitate (Yanai *et al.*, 2006). Palmitoylation of Htt at C214 by huntingtin interacting protein 14 (HIP14), a palmitoyl transferase, is reduced through a decreased interaction with mutant Htt compared with wild-type Htt. Prevention of the palmitoylation by mutating C214 increases mHtt inclusion formation.

Multiple post-translational modifications affect Htt. These are often altered in the mHtt, though most experiments have been conducted using short Htt fragments or in model systems with very long mHtt repeats. It is therefore difficult to know which of these modifications are likely to be relevant to the majority of HD patients who have glutamine tracts of 40–50 residues. Some of the modifications have been confirmed as altered in tissue from HD brains: the K444 acetylation (Jeong *et al.*, 2009) and the phosphorylation of S421 (Warby *et al.*, 2005). The normal physiological effect of the modifications is not always clear though many appear to affect Htt clearance (see below in autophagy and proteasomal clearance). It remains to be determined which of these modifications are physiologically relevant to HD in people, though they provide a number of potentially exciting drug targets.

### E. Cleavage

Proteolytic cleavage of Htt is one proposed pathogenic mechanism in HD and other neurodegenerative disorders (Wellington and Hayden, 2000). Levels or activity levels of certain proteases for which Htt is a substrate, in particular caspases, calpains, cathepsins, and matrix metalloproteinases (MMP) are increased in HD (Gafni and Ellerby, 2002; Gafni *et al.*, 2004; Hermel *et al.*, 2004; Miller *et al.*, 2010; Qin *et al.*, 2003; Sanchez Mejia and Friedlander, 2001; Silvestroni *et al.*, 2009). The cleavage products for the proteolytic cleavage sites

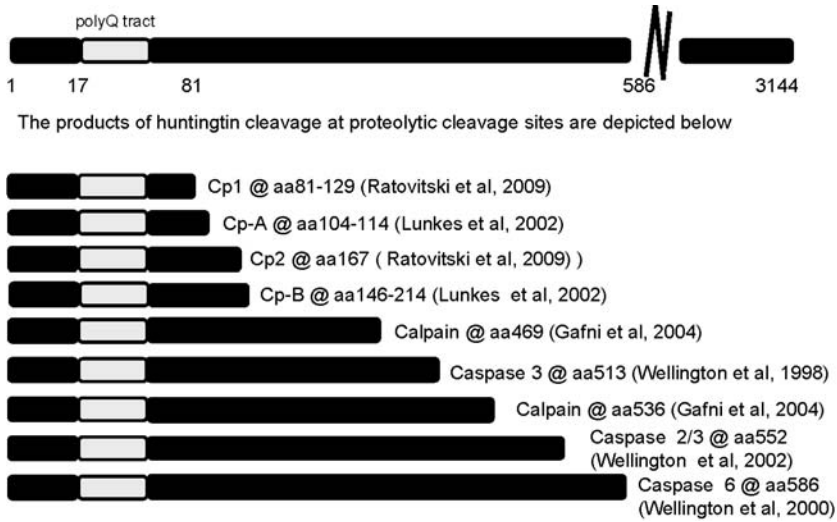


FIG. 2. Htt cleavage sites and resultant cleavage products. Mapped cleavage sites on the full length Htt protein. Note that there are more huntingtin fragments than mapped cleavage sites (for instance see Landles *et al.*, 2010)

found in Htt are shown in Fig. 2. Htt is also a substrate for a wider group of proteases including the aspartyl protease signal peptide protease-like IMP5, amino terminal signal peptide protease (SPC18), members of the secreted serine-protease kallikrein family KLK10 and KLK11, transmembrane-E3-ubiquitin ligase RNF128, and MMP2 interacting integrin ITGA2B (Miller *et al.*, 2010).

Consistent with proteolytic cleavage of Htt being a significant event in HD pathogenesis are the reports of mHtt fragments detected in cell models of HD (Lunkes and Mandel, 1998; Ratovitski *et al.*, 2007). Mouse models expressing full length mHtt have shown similar results (Gray *et al.*, 2008; Hodgson *et al.*, 1999; Tanaka *et al.*, 2006). MHtt fragments were detected in the brain of both HdhQ150 and control mice at all ages with the three smallest fragments exclusive to mutant mice (Landles *et al.*, 2010). It is worthy to note that the pattern of fragments was similar in all brain regions although the relative intensity of each was found to differ (Landles *et al.*, 2010). Small N-terminal Htt fragments were also shown in HD brains and in the cortex, striatum, and cerebellum of HD and control brain (Lunkes *et al.*, 2002). The latter fragments were compatible with caspase 3 cleaved products and could be further cleaved by calpain (Kim *et al.*, 2001).

N-terminal mHtt fragments are thought to be the toxic species in HD (Goffredo *et al.*, 2002). Nuclear inclusions can be detected with antibodies to the N-terminus of Htt only (DiFiglia *et al.*, 1997) and smaller mHtt fragments aggregate more readily (Martindale *et al.*, 1998). They are also more toxic to the cell

(Cooper *et al.*, 1998; Hackam *et al.*, 1998; Igarashi *et al.*, 2003; Martindale *et al.*, 1998). *In vivo*, transgenic mice expressing N-terminal mHtt tend to exhibit an earlier and more severe pathology and behavioral phenotype than those expressing full length mHtt (Woodman *et al.*, 2007 and reviewed in Ferrante, 2009). There is evidence that cleavage occurs before cell toxicity (Ratovitski *et al.*, 2007; Wellington *et al.*, 2002) and examination of gene expression profiles demonstrates that although mice with full-length mHtt take much longer to show a phenotype than the truncated models, such as R6/2, the profiles of downregulated genes are very similar, implying the same pathogenic process is potentiated by mHtt truncation (Kuhn *et al.*, 2007).

To gain more insight into the significance of Htt proteolysis *in vivo*, mice were generated that expressed either caspase 3 or caspase 6 resistant full length mHtt (Graham *et al.*, 2006). While no effect was shown for caspase 3, the caspase 6-resistant mice appeared to maintain normal neuronal function were devoid of striatal neurodegeneration and were spared from motor dysfunction. This implies that caspase 6 cleavage of mHtt is a potentiating event in HD pathogenesis.

Translocation of N-term mHtt fragments into the nucleus has been shown to enhance toxicity (Peters *et al.*, 1999; Saudou *et al.*, 1998) which implies that the subcellular localization of the fragments is important in HD pathogenesis. In HD patient lymphocytes, caspase cleavage occurred in the cytoplasm and the mHtt fragments sorted to perinuclear sites prior to translocation to the nucleus (Sawa *et al.*, 2005). In accordance with this, shorter mHtt fragments formed intranuclear and perinuclear aggregates, different from the exclusively perinuclear aggregates reported with longer mHtt fragments (Hackam *et al.*, 1998). Warby *et al.* (2008) observed that cleavage of htt by caspase 6 at aa586 occurred primarily in the nucleus but fragments cleaved by capase 2/3 at aa552 localized at the perinuclear region of the cell. They proposed that either the fragments are trafficked to their distinct subcellular locations after cleavage or, more likely, that the cleavage events occur at two different sites. Landles *et al.* (2010) found N-terminal fragments in the cytoplasm but not the nucleus of HdhQ150 mouse brain. So it seems Htt can enter the nucleus, but only remain there if sequestered into detergent-insoluble complexes that interfere with its nuclear export signal function or if then truncated so its nuclear export signal is removed (Truant *et al.*, 2007).

The post-translational modifications of Htt are likely to influence both its location and its susceptibility to proteolysis. Cdk5 phosphorylates Htt at Ser434 and this reduces its cleavage by caspase 3 at aa513 thereby attenuating aggregation and toxicity in a cell model of HD (Luo *et al.*, 2005). Cdk5 activity has been shown to be reduced in at least one HD transgenic mouse model (Schilling *et al.*, 1999). This leads to a lower level of Htt phosphorylation and therefore the protection against cleavage is lost. They postulate that the negative charge and possible altered structure afforded by phosphorylation could inhibit the accessibility of the caspase sites to the caspases (Luo *et al.*, 2005).

The various processes acting on the mHtt protein, post-translational modifications, cleavage, and aggregation, are clearly interlinked. We do not yet have a full picture of how these processes interact with each other, nor how that potentiates the downstream neurodegeneration of the disease. We do not understand what the toxic species of mHtt are. It is, however, clear that influencing the processes acting on mHtt such that the production of the toxic moiety is ablated is likely to be a productive pathway for therapeutic interventions.

## III. The Mutant Htt Protein and its Downstream Effects

### A. Proteasomal Dysfunction

There are two pathways for the degradation of proteins within cells. Firstly, the autophagy pathway which degrades protein complexes and organelles which would otherwise be too large for the proteasome pore (Backues and Klionsky, 2010) and secondly, the ubiquitin–proteasome system (UPS) which degrades ubiquitin-labeled short-lived, mislocated, misfolded and denatured nuclear and cytosolic proteins (Schwartz and Ciechanover, 2009). Not only is UPS dysfunction thought to play a role in neurodegenerative disease but has also been widely implicated in the pathogenesis of inflammatory disease, muscle wasting disorders, various cancers, and in hypoxia (Schwartz and Ciechanover, 2009). The possible intersection of these two processes on mHtt is shown in Fig. 3.

UPS involves a three-step process whereby a target protein is covalently conjugated to a polyubiquitin chain, recognized by the proteasome and degraded within its core (Schwartz and Ciechanover, 2009). Ubiquitin is a 76 amino acid protein conjugated to proteins by several ubiquitinating enzymes (Hochstrasser, 1996; Ortega *et al.*, 2007). hE2-25K, a ubiquitin conjugating enzyme, interacts with the first 540 amino acids of mHtt, suggestive of it being a substrate for degradation by the UPS (Kalchman *et al.*, 1996). Furthermore, a novel 5 amino acid proteasome targeting motif (FQKLL) was found within the Htt protein (Chandra *et al.*, 2008).

The observation that mHtt aggregates colocalize with ubiquitin in HD patients (DiFiglia *et al.*, 1997; Sieradzan *et al.*, 1999), HD mouse models (Davies *et al.*, 1997) and HD cellular models (Jana *et al.*, 2001; Saudou *et al.*, 1998) has implicated disruption of the UPS in HD pathogenesis. Components of the proteasome and molecular chaperones such as subunits of the 26S proteasome, HSP40, HSP70, BiP/GRP78, the RNA binding protein TIA-1, the potential chaperone 14-3-3 and α-synuclein have also been found in Htt inclusions and UPS disruption is seen in cellular HD models (Jana *et al.*, 2001; Waelter *et al.*, 2001a, 2001b; Wyttenbach *et al.*, 2000). In primary neurons established from Tet inducible HD94 mice (Yamamoto *et al.*, 2000), the truncated mHtt protein formed ubiquitin-positive

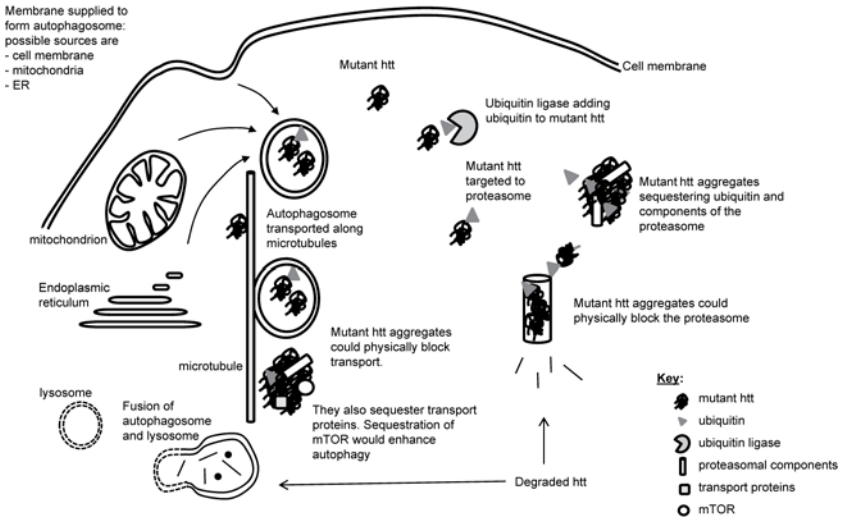


FIG. 3. MHtt may interfere with protein degradation systems and cellular transport

inclusions rapidly: upon transgene suppression, these aggregates disappeared but proteasome inhibition prevented aggregate clearance (Martin-Aparicio *et al.*, 2001). Similarly, expression of a mutant form of ubiquitin along with mHtt in cells in culture (de Pril *et al.*, 2004) and in a transgenic mouse model (de Pril *et al.*, 2010) enhanced Htt aggregate formation, though any effect on behavior or longevity of the mice was not reported. More direct evidence that the UPS is impaired in HD has come from various cellular models (Bence *et al.*, 2001; Bennett *et al.*, 2005; Duennwald and Lindquist, 2008; Hunter *et al.*, 2007; Jana *et al.*, 2001; Maynard *et al.*, 2009; Mitra *et al.*, 2009; Seo *et al.*, 2007), HD animal models (Bennett *et al.*, 2007; Wang *et al.*, 2008) and human tissue (Bennett *et al.*, 2007; Seo *et al.*, 2004).

Despite all the evidence supporting a dysfunctional UPS in HD, it should not be ignored that some studies have actually shown an increased UPS activity in HD (Bett *et al.*, 2006; Diaz-Hernandez *et al.*, 2003; Seo *et al.*, 2008) while others show no change (Bowman *et al.*, 2005; Ding *et al.*, 2002; Maynard *et al.*, 2009). This may be partly due to technical differences. In theory, mHtt could exert its effects on the UPS at any stage of the process. Firstly, the ubiquitination stage could be affected. Increased polyubiquitin levels and changes in ubiquitin linkages were noted in HD patient brains, transgenic mouse brain, and in HD cellular models (Bennett *et al.*, 2007). Levels of ubiquitin conjugates were also found to be elevated in R6/2 mouse brain though this increase in polyubiquitinated material could represent changes in cellular processes unrelated to the UPS (Maynard *et al.*, 2009). Alternatively, the UPS could become overloaded with mHtt (Bence *et al.*, 2001) or become physically obstructed by the large aggregates (Venkatraman *et al.*, 2004) (Fig. 3). It would

therefore have a reduced capacity to degrade other cellular proteins. MHtt levels would also build up within the cell, leading to a vicious cycle of UPS dysfunction. Another possibility is that mHtt aggregates sequester components of the UPS so that their availability for normal cellular functions is diminished (Jana *et al.*, 2001; Waelter *et al.*, 2001a; Wyttenbach *et al.*, 2000). The pro-apoptotic protein BimEL has been suggested as a link between impaired UPS and mHtt induced cell death as it was found to be upregulated and phoshorylated in cells expressing N-terminal mHtt (Leon *et al.*, 2010), consistent with findings in model mice (Garcia-Martinez *et al.*, 2007; Kong *et al.*, 2009; Zhang *et al.*, 2003).

It was shown that the proteasome activator, Pa28γ, could increase UPS activity in rat HD striatal cells and this appeared to promote cell survival (Seo *et al.*, 2007), making increasing UPS activity a possible therapeutic option in HD. If enhancing UPS function proved difficult, redirecting mHtt into the autophagy pathway could be explored as the two pathways could be linked (reviewed in Korolchuk *et al.*, 2010). Ubiquitin can target proteins to the autophagy pathway as well as to the UPS (Yao, 2010).

Does impaired UPS activity lead to mHtt accumulation, or is impaired UPS activity a consequence of mHtt accumulation? In single neurons tracked over their lifetime, UPS activity is more impaired in neurons that go on to form inclusions than those that do not and UPS impairment is lower in cells after inclusion formation than in those with no inclusions. Taken together, this suggests that inclusions may play a protective role in cells expressing mHtt (Mitra *et al.*, 2009) and that this is mediated by their effect on the UPS.

### B. Autophagy

In autophagy, portions of the cytoplasm are sequestered within double membrane-bound structures called autophagic vacuoles, thought to originate from the endoplasmic reticulum (Hayashi-Nishino *et al.*, 2009), mitochondria (Hailey *et al.*, 2010), or plasma membrane (Ravikumar *et al.*, 2010). They are delivered via microtubular transport to lysosomes, where subsequent fusion leads to degradation of the autophagic vacuole contents by luminal acidification, proteinase B, and the lipase, Cvt17 (Klionsky and Emr, 2000). Autophagy is a regulated process, affected by many moieties including mToR, class III phosphatidylinositol-3-kinase (Sarkar and Rubinsztein, 2008), p53 (Tasdemir *et al.*, 2008) and ubiquitin (Yao, 2010).

Autophagy has been associated with normal brain aging as well as late-onset neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, spinocerebellar ataxias, motor neuron disease, prion disease, and HD (Ventruti and Cuervo, 2007). A feature common to all these disorders is a misfolded and aggregated mutant protein, and it is thought that autophagy may be a key player in the clearance of such aggregate-prone proteins from the cell (Ravikumar *et al.*,

2008). The knock down of autophagy genes Atg5 and Atg7 in mice resulted in progressive motor deficits, cytoplasmic aggregates, and neurodegeneration (Hara *et al.*, 2006; Komatsu *et al.*, 2006). It is therefore possible that the autophagic clearance of the mHtt protein could play a part in the underlying pathogenesis of HD. There is an expansion of autophagic compartments in an HD cellular model and Htt partially colocalizes with autophagic vacuoles (Atwal and Truant, 2008; Kegel *et al.*, 2000). In addition, work in a novel HD knock-in mouse (HdhQ200) demonstrated that the autophagy-associated proteins LC3-II and p62 were increased early in the disease process (Heng *et al.*, 2010). Deletion of the polyglutamine tract from the mouse Htt protein enhances autophagy, implying that as well as being a substrate (Ravikumar *et al.*, 2002) Htt may have a modulatory role in the degradation pathway (Zheng *et al.*, 2010).

Consistent with these findings are the results from experiments pharmacologically inhibiting autophagy in HD cell models, reporting enhanced Htt aggregate formation and increased cellular toxicity (Ravikumar *et al.*, 2002). It is therefore not surprising that the converse is true when autophagy is enhanced. Stimulation of autophagy by rapamycin (inactivator of mTOR and thus an inducer of autophagy) enhanced clearance of mHtt fragments, reduced aggregates, and toxicity in a cellular model of HD (Ravikumar *et al.*, 2002). Similarly, rapamycin was shown to reduce neurodegeneration in a N171-120Q *Drosophila* model of HD and the rapamycin analog, CCI-779, was able to attenuate the phenotype in the N171-82Q mouse model of HD when administered presymptomatically. mTOR was shown to be sequestered into polyglutamine aggregates in HD cell models, transgenic mice, and patient brains which led to reduced mTOR activity and the subsequent induction of autophagy (Ravikumar *et al.*, 2004). This could explain the increased autophagosome-like structures seen in the brains of HD patients (Sapp *et al.*, 1997). Aggregates could therefore be protective, acting with mTOR to induce autophagy for self-destruction of mHtt (Sarkar and Rubinsztein, 2008).

How does mHtt interfere with the normal process of autophagic clearance within the cell? MHtt is post-translationally modified at K444 by acetylation and this has been shown to enhance the trafficking of mHtt into autophagic vacuoles thereby improving its clearance and reversing its toxic effects (Jeong *et al.*, 2009). By contrast, mHtt resistant to acetylation accumulated, resulting in neurodegeneration in cultured neurons and mouse brain. These findings suggest that acetylation of Htt is necessary for its autophagic degradation and that this is enhanced for the mutant form (Jeong *et al.*, 2009). More recently it was shown that Mhtt may interfere with the loading of cargo into autophagic vacuoles (Martinez-Vicente *et al.*, 2010)

As autophagy appears to be an integral pathway in the pathogenesis of HD, it is a potential therapeutic target for the mitigation of the adverse effects mediated by the mHtt protein on the cell. Autophagy is induced by physiological stress such as

starvation (Klionsky and Emr, 2000) and it is well established that Rapamycin is a chemical inducer of autophagy (Yang *et al.*, 2005) although the side effects it can have over long term use has triggered the search for other enhancers of mammalian autophagy. Such regulators of autophagy may prove useful in the treatment of neurodegenerative disease (Sarkar and Rubinsztein, 2008).

Furthermore, chaperone-mediated autophagy (CMA) is a selective type of autophagy that channels specific proteins labeled with HSC70 to the surface of lysosomes. It is suggested that mHtt is directed to the CMA pathway for degradation, reducing aggregates, and ameliorating symptoms in R6/2 mice (Bauer *et al.*, 2010). It may therefore be possible to search for more specific ways to selectively enhance clearance of mHtt from the cell.

### C. Transcriptional Dysfunction

Alterations in the levels of mRNAs in HD brain have been observed over many years (Arzberger *et al.*, 1997; Augood *et al.*, 1996, 1997; Norris *et al.*, 1996; Richfield *et al.*, 1995) and it has become clear that alterations in transcriptional regulation are an early pathogenic consequence of the expanded mutant HTT (Cha, 2007). The early *in situ* hybridization studies mentioned above suffered from the potential confounding effects of cell loss: it was difficult to be clear that the observed reductions in mRNAs were attributable to reduced mRNA levels rather than loss of the cells that contain those RNAs: even in early grade HD brain with very little overt pathology substantial cell loss has occurred, especially in the caudate and putamen (Vonsattel *et al.*, 1985). However, subsequent studies have confirmed substantial reductions in specific mRNAs in human brain (Hodges *et al.*, 2006) and mouse model brain (Luthi-Carter *et al.*, 2000, 2002a, 2002b) (Kuhn *et al.*, 2007; Thomas *et al.*, 2011). What all of these studies examine is the steady state level of specific RNAs in the brain and this is a reflection of dynamic processes affecting RNA: synthesis, stability, and degradation. In most cases, we do not know which of these processes acting on RNA are important in mediating the altered levels observed, but possible molecular mechanisms that might underlie these observations have also been the subject of a detailed study (Cha, 2007).

Subsequent to a number of candidate gene expression analyses in mouse models (see Cha, 2007), the advent of whole genome microarray analysis to analyze all RNAs from a cell or tissue in parallel allowed a global overview of gene expression changes in HD models and in HD brain, usually referred to as expression profiling (Chan *et al.*, 2002; Hodges *et al.*, 2006; Luthi-Carter *et al.*, 2000, 2002a, 2002b; Kuhn *et al.*, 2007; Thomas *et al.*, 2011; Zucker *et al.*, 2005). These studies confirmed the initial view that transcriptional changes were an early event in the disease and showed that the changes in a number of different mouse model striata looked very like those in human caudate (Kuhn *et al.*, 2007). While these

studies are also affected by the issue of cell loss, laser capture microdissection has demonstrated in HD brain (Hodges *et al.*, 2006) and in mouse brain (Sadri-Vakili *et al.*, 2006; Zucker *et al.*, 2005) that downregulated gene expression is cell autonomous and reflects mRNA complements of neurons rather than other cell types. Expression profiling in cell culture models also supports this conclusion (Kita *et al.*, 2002; Runne *et al.*, 2008; Sipione *et al.*, 2002).

There is a greater concordance in the genes with lowered expression in HD across human HD caudate and mouse model striata (Hodges *et al.*, 2006; Kuhn *et al.*, 2007) than in the upregulated genes. There is also a significant overlap of genes altered in expression between brain regions (Hodges *et al.*, 2006). Many of the pathways implicated by mRNA changes in caudate relate to neuronal signaling and homeostasis (Hodges *et al.*, 2006; Luthi-Carter *et al.*, 2000). The majority of changes in these categories have lower expression in HD. The most significant group of mRNAs showing changed expression in the caudate encode metabotropic and ionotropic receptor subunits and those conveying signals from different transmitters, including excitatory amino acids, GABA, dopamine, and cannabinoids.

The transcriptional changes seen in brain are also reflected in other tissues, implicating this as a fundamental property of cells expressing mHtt. Muscle from both HD subjects and several mouse lines (Luthi-Carter *et al.*, 2002b; Strand *et al.*, 2005) show a similar altered gene expression signature, which indicated a switch from fast twitch to slow twitch muscle fibers.

The mechanism by which these alterations in mRNA levels occur has been investigated extensively. Htt interacts with a number of transcriptionally active proteins: the nuclear receptor repressor NCoR (Boutell *et al.*, 1999), CREB-binding protein (CBP) (Nucifora *et al.*, 2001; Steffan *et al.*, 2000), TATA-binding protein (TBP) (Huang *et al.*, 1998), TAFII130 (Dunah *et al.*, 2002; Shimohata *et al.*, 2000), p53 (Bae *et al.*, 2005; Steffan *et al.*, 2000), Sp1 (Dunah *et al.*, 2002; Ravache *et al.*, 2010), repressor element 1 transcription factor REST (Zuccato *et al.*, 2003). In addition, mHtt binds the promoter of the peroxisome proliferator activated receptor γ coactivator 1 α (PPARγ) PGC-1α preventing CREB/TAF4 activation and thus inhibits its expression (Cui *et al.*, 2006) which may explain some of the mitochondrial effects seen in HD (see below). Although some of these interacting proteins have been detected in the Htt-positive inclusions, this does not appear to be the mechanism by which they mediate alterations in mRNA levels: cells with and without inclusions demonstrate reduced RNA levels in R6/2 striatal neurons (Sadri-Vakili *et al.*, 2006).

Expression profiles are one way of examining the effect of treatments in disease. This is most helpful if accessible tissues can be profiled but profiles in blood of HD patients have so far proved to be inconsistent (Borovecki *et al.*, 2005; Runne *et al.*, 2007) so using them as a biomarker for treatment trials is not currently feasible. Some preclinical treatment studies in HD mice that have examined brain gene expression after treatment have found that the gene expression changes have

been reversed (Hockly *et al.*, 2003; Morton *et al.*, 2005; Pallos *et al.*, 2008; Steffan *et al.*, 2001; Thomas *et al.*, 2008). Some of these treatments were histone deacetylase inhibitors which would be expected to target repression of transcription directly but treatments that would not be predicted to have this effect also demonstrate gene expression changes that appear more like controls (Morton *et al.*, 2005).

As gene expression profiling reveals steady state mRNA levels the dynamics of mRNA synthesis and degradation remain unclear. The transport and local translation of mRNAs in dendrites close to synapses have been shown to be affected by Htt (Savas *et al.*, 2010) and this too may alter the concentrations of RNA by increasing or reducing the stability and longevity of specific RNAs.

### D. Transport Defects

The movement of cargo along cytoskeletal tracks forms the basis of the cellular transport system. In general, actin is used for short-range transport and microtubules are involved in more long-range transport (Caviston and Holzbaur, 2009). Neurons are polarized cells with transport away from and toward the cell body defined as anterograde and retrograde transport, respectively. The motor proteins dynein and kinesins are involved with microtubule-mediated transport, whereas the myosins associate with actin filaments. Dynein is involved in retrograde transport and the kinesins are key in anterograde transport. These trafficking processes are required for neuronal differentiation and survival, hence the described association between defective motor proteins and neurological disease (Salinas *et al.*, 2008).

The first suggestion of a role for Htt in vesicular trafficking came from biochemical studies showing that wild-type Htt was associated with vesicle-rich fractions (DiFiglia *et al.*, 1995). Since then, a growing body of evidence has further supported this notion. Htt interacts with several proteins involved in vesicular trafficking. These include HAP1 (Li *et al.*, 1995), HIP1 (Kalchman *et al.*, 1997; Wanker *et al.*, 1997), HIP14 (Singaraja *et al.*, 2002), HAP40 (Peters and Ross, 2001), PACSIN1 (Modregger *et al.*, 2002), and proteins involved in SNARE-mediated vesicle fusion (Kaltenbach *et al.*, 2007). Most of these were shown to have altered interaction with the mutant protein. The expression of several trafficking proteins was found to be affected in early HD as shown by microarray analysis of Htt-inducible striatal cells (Sipione *et al.*, 2002), and pathways revealed by gene profiling in human HD cortex included microtubule-based movement and vesicle transport (Hodges *et al.*, 2006). Direct experimental evidence *in vitro* and *in vivo* demonstrates perturbed axonal transport by mHtt (Gunawardena *et al.*, 2003; Sinadinos *et al.*, 2009; Szebenyi *et al.*, 2003). Aggregates of mHtt were reported in the cytoplasm and neuronal processes of a transgenic HD *Drosophila* model which increased over time and were shown to block axonal transport (Lee *et al.*, 2004). Axonal transport defects in *Drosophila* were not exclusive to

Htt, but also seen for other pathogenic polyglutamine proteins and were shown to be polyglutamine-length dependent (Gunawardena *et al.*, 2003).

Htt binds directly to dynein and facilitates vesicle motility along microtubules (Caviston and Holzbaur, 2009) and the organization of the Golgi apparatus was dependent on wild-type Htt with its dynein and HAP1 interacting domains intact (Pardo *et al.*, 2010). Htt also enhances vesicular transport of BDNF along microtubules in neuronal cell lines from knock-in Q109/Q109 HD mice in association with HAP1 and the $p150^{glued}$ subunit of dynactin (Gauthier *et al.*, 2004).

Trafficking defects have also resulted from reducing levels of wild-type Htt. Depletion of endogenous Htt disrupted both anterograde and retrograde axonal transport of amyloid precursor protein (APP) in primary mouse cortical neurons (Her and Goldstein, 2008) and attenuated BDNF transport in mouse neuronal cells (Zala *et al.*, 2008). Reduction of endogenous wild-type Htt to less than 50% of normal levels caused trafficking defects in mouse striatal neurons and interestingly, mHtt could partially restore this defect (Trushina *et al.*, 2004). Transport defects defined by organelle accumulation were revealed in *Drosophila* expressing reduced levels of Htt (Gunawardena *et al.*, 2003). These findings might explain the neurological phenotypes seen in mice with reduced levels of normal Htt (Auerbach *et al.*, 2001).

When primary embryonic striatal neurons were isolated from control and transgenic mice expressing full length Htt with either 16Q or 72Q, the speed of vesicular transport was found to be reduced in the mutant neurons. In particular, mitochondria moved more slowly and stopped more frequently along their course in mHtt-expressing neurons (Trushina *et al.*, 2004). The impaired trafficking was recapitulated *in vivo*, in 5-month old transgenic mice expressing full length Htt with 72Q, prior to the onset of symptoms. BDNF transport was found to be attenuated in neuronal cells derived from HD knock-in mice (Gauthier *et al.*, 2004). Similarly, APP and BDNF transport was impaired in Q150 presymptomatic mice (Her and Goldstein, 2008). It was also shown that while mHtt was not capable of stimulating BDNF-labeled vesicles in mouse neuronal cells, this dysfunction could be rescued by phosphorylating Htt at Ser 421 (Zala *et al.*, 2008). Phosphorylation at Ser421 can act as a "directional switch" resulting in a net change in direction from retrograde to anterograde transport of vesicles along microtubules caused by the phosphorylated form of Htt recruiting more kinesin to vesicles (Colin *et al.*, 2008).

How might mHtt interfere with cellular transport? MHtt aggregates could directly block axonal transport due to their sheer size, as electron and confocal microscopy have revealed aggregates large enough to occupy the entire cross-section of the axon (Lee *et al.*, 2004; Li *et al.*, 2003a). Indeed, mHtt aggregates have been likened to "physical roadblocks" for mitochondria transport in cortical neurons (Chang *et al.*, 2006). Another possibility is that aggregated Htt sequesters proteins required for vesicle transport, depleting the soluble pools of these proteins

and rendering them unavailable (Caviston and Holzbaur, 2009; Gunawardena *et al.*, 2003; Qin *et al.*, 2004; Trushina *et al.*, 2004) (Fig. 3).

Aberrant Htt interactions could play a role in defective transport. Htt associates with microtubules and reduces transport efficiency (Gutekunst *et al.*, 1995; Hoffner *et al.*, 2002; Smith *et al.*, 2009; Trushina *et al.*, 2003). HAP40 is an Htt-interacting protein implicated in endosome motility and the interaction appears to mediate a shift from long-range microtubule-based transport to short-range actin-based transport (Pal *et al.*, 2006). MHtt is palmitoylated by HIP14 (Singaraja *et al.*, 2002), but palmitoylation of the mutant form is reduced potentially leading to disruption of trafficking processes in the cell (Yanai *et al.*, 2006). The interaction between mHtt and HAP1 disrupted the development of trafficking complexes which could contribute to the trafficking defects observed in HD (Gauthier *et al.*, 2004). Htt may also inhibit fast axonal transport indirectly through upregulation of cJun N-terminal kinase 3 (JNK3) activity. JNK3 phosphorylates Ser176 of kinesin heavy chain which inhibits its binding to microtubules, thus suggesting an explanation for the impaired fast axonal transport reported in HD (Morfini *et al.*, 2009).

Trafficking defects could affect the cell in several ways. They could lead to loss of neurotrophic support for the cell due to defective BDNF transport (Gauthier *et al.*, 2004) and alter neurite outgrowth and maintenance (Trushina *et al.*, 2004). Aberrant trafficking of mitochondria could affect cell survival through an inability to meet the energy demands of the cell, disruption to calcium signaling, and the accumulation of damaged mitochondria that would have otherwise been transported to their site of degradation in the cell (Chang *et al.*, 2006). GABA(A) receptors are trafficked to synapses by kinesin motor protein 5 (KIF5) via HAP1. MHtt reduces GABA(A) receptor transport with a consequent reduction in inhibitory synaptic currents in neuronal cells derived from 109Q/109Q knock-in mice (Twelvetrees *et al.*, 2010). The functional consequence of this could be that impaired trafficking might enhance neuronal excitability, a well-documented feature of HD (Cepeda *et al.*, 2007).

### E. Energy Metabolism

Defective energy metabolism has been suspected of a role in HD pathogenesis for several decades because of the progressive weight loss (Djousse *et al.*, 2002; Mahant *et al.*, 2003) and the metabolic alterations that can be seen in both brain (Jenkins *et al.*, 1993; Reynolds *et al.*, 2005; Sanchez-Pernaute *et al.*, 1999) and muscle (Lodi *et al.*, 2000) in patients. These observations have been followed up by investigations of specific energy pathways in cell and animal models (see Oliveira, 2010 for a recent review) and by more detailed studies in patients, including therapies aimed at correcting energy metabolism deficiencies (Huntington Study Group, 2001).

Various imaging modalities have implicated altered energy metabolism in HD brain. Low resolution 1H magnetic resonance spectroscopy (MRS) studies have shown increased *N*-acetyl aspartate (NAA) in HD basal ganglia (Reynolds *et al.*, 2005; Sanchez-Pernaute *et al.*, 1999): NAA is thought to mark mitochondrial loss or neuronal dysfunction (Clarke *et al.*, 1998). More directly related to energy metabolism, raised lactate and decreased creatine levels have been observed in HD brain (Jenkins *et al.*, 1993; Reynolds *et al.*, 2005). 18Fluoro-deoxyglucose positron emission tomography (PET) studies have also identified defects in energy metabolism in the striata of both symptomatic and presymptomatic HD patients (Kuwert *et al.*, 1990, 1993; Martin *et al.*, 1992). All of these observations are compromised to some extent by the neuronal atrophy known to occur in HD (Vonsattel *et al.*, 1985) which may interfere with the imaging results. Recent detailed morphometric imaging studies have demonstrated that atrophy of the caudate in presymptomatic HD gene-positive subjects more than 10 years before estimated clinical onset of disease (Tabrizi *et al.*, 2011).

However, examination of energy metabolism in more easily accessible patient tissues and in post-mortem brain, have also demonstrated defects in HD. Muscle, lymphoblasts and platelets have shown altered oxidative phosphorylation (Arenas *et al.*, 1998; Parker *et al.*, 1990; Sawa *et al.*, 1999; Turner *et al.*, 2007) with the most consistent evidence pointing to a defect in mitochondrial complex II/III activity. Studies in HD mouse models and cell lines have provided some partial support for these hypotheses though there are many inconsistent data.

Complex IV activity was altered in symptomatic R6/2 striatum and cortex (Tabrizi *et al.*, 2000) but no such defects in mitochondrial oxidative phosphorylation were detected in the brains from a series of genetic mouse models of HD, all with longer time courses to phenotype development than R6/2, assayed both before and after the development of any phenotype (Browne, 2008; Guidetti *et al.*, 2001; Lee *et al.*, 2007; Milakovic and Johnson, 2005). Cell lines and striatal tissue from the HdhQ111 animal models have demonstrated altered cAMP (Gines *et al.*, 2003) and ATP (Gines *et al.*, 2003; Milakovic and Johnson, 2005; Seong *et al.*, 2005) levels though these appear to be a result of altered mitochondrial $Ca^{2+}$ fluxes rather than direct effects on the electron transport chain (Choo *et al.*, 2004; Milakovic and Johnson, 2005; Panov *et al.*, 2002; Seong *et al.*, 2005). The reduced mitochondrial $Ca^{2+}$ has been shown to lead to activation of the mitochondrial transition pore with an increased permeability of the inner membrane to small ions and a collapse of the membrane potential which prevents ATP production (Panov *et al.*, 2002). *In vitro* incubating mHtt with mitochondria also produces this effect (Choo *et al.*, 2004; Panov *et al.*, 2002). In addition, proteomic analysis in knock in mouse lines also gave more changes in mitochondrial proteins than was expected and as these included both increases and decreases in individual proteins of the mitochondria this implies a shift in mitochondrial metabolism rather than a straightforward alteration in mitochondrial numbers (Deschepper *et al.*, 2011).

Oliveira (Oliveira, 2010; Oliveira *et al.*, 2007) notes that the precise experimental technique used can influence observations of the respiratory chain and may well account for some of the contradictory results of these experiments.

One of the transcriptional defects seen in models of HD, as noted above, is a reduction in levels of the transcriptional coactivator PGC-1a mRNA (Cui *et al.*, 2006): PGC-1a regulates mitochondrial biogenesis, respiration, and density in neurons (Leone *et al.*, 2005; Lin *et al.*, 2002; Wareski *et al.*, 2009). HD mutant mice show altered thermoregulation and both the mice and patients had reduced expression of PGC-1a target genes in the striatum (Chaturvedi *et al.*, 2009; Weydt *et al.*, 2006). Sequence variants in PPARGC1A, the gene encoding PGC-1a, are also associated with altered age-at-onset of HD further implying a role for mitochondrial events in HD development (Che *et al.*, 2011; Weydt *et al.*, 2009).

Defects in energy metabolism are clearly part of the clinical phenotype of HD. Dissecting the molecular origins of the effects seen by brain imaging in people has not provided a clear mechanism as to the nature of these defects. It seems most likely that these changes in energy metabolism are secondary to other molecular events in HD, but if they are important in pathogenesis, ameliorating them should be therapeutically beneficial and there is some evidence in models and in HD patients that this might be the case.

## F. Excitotoxicity

Excitotoxicity refers to the death of neurons after overstimulation by excitatory neurotransmitters and has been proposed as a toxic mechanism in HD for several decades (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). The most vulnerable cells in HD are the medium spiny neurons of the caudate and putamen and these cells' main input is of excitatory glutamate from the cortex. It has been known for some time that these cells are particularly susceptible to excitation and that the disease could be mimicked using glutamate analogues in the striata such as quinolinic and kainic acid (McGeer and McGeer, 1976; Schwarcz *et al.*, 1984). It is not clear how excitotoxicity is mediated by processes, discussed above, that are affected by mHtt, but there is some evidence indicating that excitotoxicity does occur and that it may well be important in the final fate of neurons, particularly in the striatum (see for instance Estrada Sanchez *et al.*, 2008 for a detailed review). Remacemide, an NMDA receptor antagonist, has beneficial effects on the phenotype of the R6/2 mice (Ferrante *et al.*, 2002) and various NMDA receptor antagonists have been used in other mouse models (Beal and Ferrante, 2004; Schiefer *et al.*, 2002) and in HD subjects (Mestre *et al.*, 2009).

Glutamate activates a number of neurotransmitter channels that allow ion influx, including NMDA, AMPA, and kainic acid receptors as well as a series of metabotropic receptors that activate intracellular G-protein signaling cascades. NMDA receptors transport $Ca^{2+}$ ions and AMPA receptors $Na^{+}$ ions. Glutamate levels are

controlled by a series of glutamate transporters found on both neuronal and glial cells, which rely on the cells' $Na^+/K^+$ gradient, and are therefore heavily dependent on oxidative energy production to maintain the $K^+$ gradient inside the cells through $Na^+/K^+$ ATPase (Estrada Sanchez *et al.*, 2008). The observed excitotoxicity is mediated by the rise in $Ca^{2+}$ transported into cells through the overstimulated NMDA receptors which in turn activates a series of enzymes that lead eventually to cell death. Thus, the alterations in energy metabolism discussed above might well predispose neurons to excitotoxic cell death in HD, and the glutamate input would explain the specific susceptibility of the striatal medium spiny neurons to such damage.

NMDA expressing neurons are lost from human HD striatal tissue early in disease (Albin *et al.*, 1990; Young *et al.*, 1988). In human HD brain, the expression of the NMDA subunits NR1 and NR2A is substantially downregulated in the caudate but not in cerebellum or cortex and the AMPA receptor subunits GluR1, GluR2, and GluR3 are all similarly downregulated in the caudate (Hodges *et al.*, 2006). Of the kainate receptors, only Gria2 and 5 are downregulated in caudate and the metabotropic glutamate receptors are much less significantly downregulated. This may be a result of shifts in the cell populations as medium spiny neurons die in the caudate, though LCM and cellular models indicate that some of these changes are cell autonomous (Hodges *et al.*, 2006; Runne *et al.*, 2008). Examination of similar studies in mouse models also show some concordant changes in striata (Ali and Levine, 2006; Hodges *et al.*, 2008; Luthi-Carter *et al.*, 2000, 2002a, 2002b). Dissociated striatal neurons from R6/2 mice had a decreased proportion of cells expressing the NR2A subunit which would potentially account for its reduced expression (Ali and Levine, 2006).

However, the most compelling evidence that excitotoxicity is relevant to HD pathology comes from mouse model studies using NMDA receptor agonists or showing increased NMDA currents (Cepeda *et al.*, 2001; Chen *et al.*, 1999; Fan and Raymond, 2007; Levine *et al.*, 1999; Li *et al.*, 2003b, 2004; Milnerwood *et al.*, 2010; Shehadeh *et al.*, 2006; Zeron *et al.*, 2001, 2002, 2004). Enhanced NMDA-mediated $Ca^{2+}$ currents were observed in striatal neurons of both R6/2 and YAC72 mice (Cepeda *et al.*, 2001; Li *et al.*, 2004) and this is linked to apoptosis of YAC72 dissociated medium spiny neurons (Shehadeh *et al.*, 2006). Potentiation of the NMDA-induced $Ca^{2+}$ current had a greater effect on subsequent excitotoxicity in these cells than induction of mitochondrial stress (Shehadeh *et al.*, 2006). However, it has also been found that the R6/1 and R6/2 lines of HD mutant mice are more resistant to excitotoxic insults than wild-type mice (Hansson *et al.*, 1999; MacGibbon *et al.*, 2002; Morton and Leavens, 2000). Why this should be the case is unknown but indicates the importance of more research to elucidate the role of excitotoxicity as a final mechanism of cell death in HD.

Quinolinic acid, an excitotoxin used to produce rodent models that mimic HD, is a metabolite in the catabolism of tryptophan (see Schwarcz *et al.*, 2010). In yeast, a disabling mutation of the gene encoding kynurenine 3-monooxygenase (KMO), an enzyme in the tryptophan catabolic pathway, was found to suppress

the toxic effects of a mHtt fragment (Giorgini *et al.*, 2005). 3-Hydroxykynurenine (3-HK), the immediate product of KMO and quinolinate, further down the catabolic pathway, was absent in this yeast strain. 3-HK and quinolinate were found to be raised in low grade HD brain (Guidetti *et al.*, 2004) and in several different mouse models one or both of these metabolites were also raised, though fairly late in phenotype development (Guidetti *et al.*, 2006; Giorgini *et al.*, 2008). These results make KMO a therapeutic target in HD and a kynurenic acid analog ameliorated the behavioral phenotype and extended the lifespan of an N-terminal mHtt mouse model (Zadori *et al.*, 2011) indicating that this avenue is well worth further exploration as a drug target.

## IV. Conclusions

There are multiple possible pathogenic pathways operating in HD. It is unclear which of those are most important in precipitating disease, though clearly work modifying the mutant RNA and protein offers a path to treatments that might preclude the need to understand all the downstream effects of the mutation. However, factors operating on the mutant protein itself, such as cleavage, post-translational modification, and other factors that lead to conformational changes and possibly aggregation are likely to be important in pathogenesis and it is not clear when those modifications occur in people carrying a mutant *HTT* gene. The downstream pathways affected by mHtt are also manifold and there is substantial evidence to support roles in pathogenesis for each of them. While this renders it difficult to know the most important pathogenic pathways in disease manifestation and progression, it does provide a number of potential avenues for possible treatments. HD is caused by essentially a single mutation in all sufferers, but nevertheless the relationship between that mutation and the downstream consequences has still to be clarified.

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# EXPERIMENTAL MODELS OF HD AND REFLECTION ON THERAPEUTIC STRATEGIES

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Huntington's disease (HD) is an autosomal dominant, progressive, and fatal neurodegenerative disorder caused by an expanded polyglutamine cytosine–adenine–guanine repeat in the gene coding for the protein huntingtin. Despite great progress over the past two decades since the identification of the gene mutation, a direct causative pathway from the HD gene mutation to neuronal dysfunction and death has not yet been established. One important advance in understanding the pathogenic mechanisms of this disease has been the

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00016-X




0074-7742/10 $35.00

development of experimental mouse models that replicate many of the clinical, neuropathological, and molecular events in HD patients. These murine models have played a critical role in providing accurate and experimentally accessible systems to study multiple features of disease pathogenesis and to test potential therapeutic strategies. A better understanding of the pathophysiological mechanisms of disease and how they interrelate has become important in identifying a treatment for HD and in the design of human clinical trials. In this chapter, we review the current state of HD mouse models and their successes in elucidating disease pathogenesis and in developing pharmacotherapies. There is no clinically proven treatment for HD that can halt or ameliorate the inexorable disease progression. As such, a guide to assessing studies in mouse models and salient issues related to translation from mice to humans are included.

## I. Introduction

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder that is characterized clinically by progressive cognitive and memory impairments, heightened irritability, depression, weight loss, and choreic motor abnormalities (Huntington, 1872). HD occurs worldwide in all races and ethnicities with a prevalence of 5–10 per 100,000. In the United States, ~30,000 individuals are affected, and an additional 150,000 are genetically at risk for developing the disease. The age of onset is typically within the fourth decade; however, the disease can manifest at any age from infancy to the ninth decade (Kremer *et al.*, 1994). The juvenile form of the disease (Westphal variant), found in 2% of the diseased population, occurs in patients younger than 20 years of age and represents a distinct clinical entity of HD characterized by a rapidly coursing hypokinetic syndrome (Jervis, 1963). Once symptomatic, individuals affected with adult onset HD experience early functional decline and require increasing care and supervision for another 15 to 25 years with pneumonia and hypertrophic cardiomyopathy as the most frequent causes of death. As such, HD disproportionately consumes medical, social, and family resources (Helder *et al.*, 2001).

While HD was initially suggested to be chronic encephalitis, in 1872 George Huntington gave a detailed description of the disease, based on the description of affected patients from his father's and grandfather's practice in East Hampton, Long Island, NY. These HD patients could be traced from a small number of individuals having emigrated from Suffolk, England, in 1630 (Bruyn *et al.*, 1979). In 1908, Jergelsma first described the characteristic neuropathological alterations within the basal ganglia (Jergelsma, 1908). It was not until 1985 that a thorough description of the anatomical and histopathological changes was reported by

Vonsattel and colleagues, topographically grading gross atrophy and the severity of striatal neuron loss and increased astrogliosis within the neostriatum (Vonsattel *et al.*, 1985). Basal ganglia neurodegeneration has been the most thoroughly characterized pathological event of HD and has been central to both the development of animal models and the formation of hypotheses involving chorea and potential mechanisms of neuronal death. Although neuropathological changes are seen prior to symptomatic clinical manifestation, they are specific to the subcortical white matter (Rosas *et al.*, 2006, 2008). While many brain regions are affected by HD, the most prominent neuropathological feature of manifest HD is marked gross atrophy of the neostriatum with concomitant neuronal degeneration and astrogliosis within the caudate nucleus and putamen. There is a topographic progression of neuronal loss and astrogliosis, which is first observed in the dorso-medial aspect of the striatum that progresses ventro-laterally, with relative sparing of the ventral striatum (Vonsattel *et al.*, 1985). The hallmark of HD is selective neuronal degeneration in which medium-sized spiny, gamma aminobutyric acid striatal projection-neurons are affected early and most severely, while large cholinergic neurons and medium-sized nicotinic adenine dinucleotide phosphate (NADPH)-diaphorase aspiny neurons are relatively spared (Ferrante *et al.*, 1985, 1986, 1987). Additionally, there is a reduction in striatal neurochemicals associated with select neuronal subtypes that parallels the observed striatal neurodegeneration (Bird and Iversen, 1974), with enkephalin-expressing striatal projection neurons appearing more vulnerable in comparison to substance-P striatal neurons (Reiner *et al.*, 1988). HD is a noncell autonomous disease. Despite the fact that medium-sized spiny neurons are the primary neostriatal target in HD, astroglia may also play a significant role in neuronal loss. It had been long suggested that glia, mainly astrocytes, reflect a response to neuronal dysfunction and death. It has been recently reported that astroglia in HD, via alterations in their glutamate transporters, may contribute to the pathogenesis of the disease (Faideau *et al.*, 2010).

The gene responsible for HD was identified and cloned in 1993 (MacDonald *et al.*, 1993). Although there has been great progress in understanding the clinical and molecular phenomena associated with HD, a direct causative pathway from the HD gene mutation to neuronal dysfunction and death has not yet been established in the subsequent 18 years. This gene normally codes for huntingtin (Htt), a large, highly conserved protein whose normal function is thought to be involved in fast axonal transport, specifically enhancing vesicular transport of brain-derived neurotrophic factor along microtubules (Gauthier *et al.*, 2004). HD patients have an expanded polymorphic trinucleotide cytosine-adenine-guanine (CAG) repeat near the 5' end of on the short arm of chromosome 4, producing mutant Huntingtin (mHtt). Individuals with HD express both the normal and the mutant alleles. Normal individuals have 17–29 CAG repeats, while individuals with HD have more than 38 repeats. Once expanded into the pathogenic range, the

number of repeats has an inverse relationship with the age of onset and the severity of the disease (Djousse *et al.*, 2003). Mutant Htt expression is observed throughout the body and in all areas of the brain within both neurons and astrocytes (DiFiglia *et al.*, 1995, 1997, Kuemmerle *et al.*, 1999) and is cleaved during proteolysis, releasing an N-terminal fragment containing the expanded polyglutamine amino acid sequence. This fragment forms aggregates with itself that accumulate in both the cytoplasm and the nucleus, as well as neuronal arbors (Kuemmerle *et al.*, 1999).

The pathogenic significance of cytosolic and nuclear mutant huntingtin aggregation remains unclear. Ongoing debate continues, as with other neurodegenerative disorders in which protein aggregates are a hallmark of disease, questioning whether inclusions formed by the aggregated N-terminal truncation of huntingtin cause neuronal death through alterations of nuclear transport or DNA arrangements affecting transcription (Cha, 2000), or whether they represent the sequestration of the mutant protein impeding polyglutamine-induced neurotoxicity (Klement *et al.*, 1998; Kuemmerle *et al.*, 1999; Ordway *et al.*, 1997; Rosas *et al.*, 2006; Saudou *et al.*, 1998). There is evidence supporting both sides. In transgene models, nuclear huntingtin and ubiquitin aggregation is present prior to the formation of neurological deficits, implicating aggregation in neuronal dysfunction and subsequent neuronal death (Davies *et al.*, 1998), (Meade *et al.*, 2002). On the other hand, "shortstop" YAC transgenic mice have widespread nuclear huntingtin inclusions, but do not demonstrate a significant HD phenotype (Slow *et al.*, 2005), a finding that supports the cytoprotective hypothesis (Zhang *et al.*, 2008). As such, huntingtin inclusions may not be a key player in the pathogenic events leading to selective neuronal death nor a predictor of neuronal death. It may well be that soluble mHtt fragments cause the pathological interactions and subsequent neuronal death, although it seems logical to reason that the many pathophysiological events that result from protein aggregation, including the sequestration of essential cellular transcription factors and neuronal proteins resulting in decreased function (Nucifora *et al.*, 2001), altered proteosomal functions (Bence *et al.*, 2001), and the localization of mHtt aggregates to organelles such as mitochondria (Panov *et al.*, 2002a), have deleterious effects on the cell. The mass effect of mtHtt aggregates may also physically impede mitochondrial dynamics of fusion and fission (Kim *et al.*, 2010a). In further support of this, therapies that reduce huntingtin aggregates have been shown to significantly ameliorate the behavioral and neuropathological phenotype when administered in HD mouse models (Beal and Ferrante, 2004; Stack and Ferrante, 2007).

There is no proven therapy to either delay the onset or slow the progression of HD. Current clinical treatment focuses on symptom management and includes a number of drug agents used to treat the motor and behavioral changes associated with HD. While great strides have been made in understanding the pathogenesis of HD, there is still an incomplete understanding of the disease mechanisms. Animal toxin and genetic models that closely mimic the neurobiological and clinical

symptoms of the disease may provide an alternative approach for the study of HD molecular pathogenesis, in the development of existing treatments and novel therapeutic strategies for HD, and in providing insight into peripheral and central nervous system biomarkers of disease. Therefore, animal models are a crucial part of the rapidly advancing field of HD research. In this chapter, we review experimental models of HD and their varied utilities for understanding the disease mechanisms and associated therapeutic strategies related to translation from mice to humans.

## II. Mouse Models of HD

### A. Toxin Models of HD

#### 1. *Excitotoxin lesions in animals*

Suitable animal models of HD must at least replicate the neuropathological features of the disorder. There is increasing evidence suggesting that excitotoxicity plays a prominentrole in both acute and chronic neurological diseases. As such, one of the earliest experimental murine models established for HD included the direct administration of excitatory agonists into the central nervous system. The first observations arguing that excitotoxicity may play a role in HD were made by the McGeers and Coyle and Schwarcz (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Each group of investigators showed that injections of the excitatory glutamate-type neurotoxin, kainic acid, produced degeneration of striatal GABAergic projection neurons with preservation of striatal afferents, resembling the neuropathological findings in HD. Subsequent use of *N*-methyl-D-aspartate (NMDA)-type excitotoxins, including quinolinic acid, refined the model. While kainic acid did not selectively affect striatal neurons, quinolinic acid that showed differential sparing of striatal neurons. Quinolinic acid alters both GABAergic and substance P-containing neurons, with relative sparing of NADPH-diaphorase and cholinergic neurons, that latter of which are known to be spared in HD (Ferrante *et al.*, 1985, 1987). Quinolinic acid administration also results in an age-dependent decrease in enkephalin neuron vulnerability in contrast to substance P striatal neurons (Sun *et al.*, 2003) and produces a more accurate model of HD (Beal *et al.*, 1991a; Ferrante *et al.*, 1993). Other NMDA agonists also reproduce relative sparing of NADPH-diaphorase neurons (Beal *et al.*, 1988). Chronic quinolinic acid lesions, where months have passed to allow resorption of the necrotic injection site, closely reproduce the patterns of selective neuronal sparing in the rat striatum and cerebral cortex observed in human HD patients (Beal *et al.*, 1991b). In addition,

quinolinic acid lesions in the monkey provide an experimental primate model that closely resembles the neuropathological, neurochemical, and clinical features of human HD, showing a disproportionate involvement of the matrix compartment similar to that seen in HD patients (Ferrante *et al.*, 1993). These changes were accompanied by behavioral alterations, suggesting a hyperkinetic movement disorder. Dopaminergic agonist-inducible chorea was observed in the primates and was indistinguishable from that seen in HD subjects (Storey *et al.*, 1994). Ultrastructural analysis confirmed axon-sparing lesions with neuronal loss and astrogliosis. These animal models demonstrate a characteristic profile consistent with the features of HD and strongly support the hypothesis that excitotoxicity plays a role in the pathogenesis of HD.

While excitotoxic lesions in the striatum are dependent upon corticostriatal glutamatergic inputs (Biziere and Coyle, 1979; McGeer *et al.*, 1978), there is also substantial evidence showing that striatal excitotoxic lesions are dependent on substantia nigra dopaminergic inputs (Biziere and Coyle, 1979; McGeer *et al.*, 1978; Meldrum *et al.*, 2001). Nigrostriatal dopaminergic neurotransmission is altered in HD and may contribute to striatal vulnerability, as released dopamine can act as a stressor against striatal neurons through oxidative mechanisms, as well as modulate glutamate release (Jakel and Maragos, 2000; Reynolds *et al.*, 1998; Maragos *et al.*, 1998). It is possible that both cortical and nigral afferent inputs may be responsible for the regional selectivity of neuronal degeneration observed in HD (Stack *et al.*, 2007a). Interestingly, deafferentation of both the corticostriatal and nigrostriatalpathways mitigate striatal stress and neurodegeneration in the R6/2 HD mouse model (Stack *et al.*, 2007a). Both surgical and chemical lesions of the corticostriatal and nigrostriatal pathways, respectively, improved the behavioral, neuropathological, and biochemical phenotype in R6/2 transgenic mice and extend survival (Stack *et al.*, 2007a). Decortication ameliorated hindlimb clasping, striatal neuron atrophy, and huntingtin-positive aggregates, improving *N*-acetyl aspartate/creatine levels, reducing oxidative stress, and significantly lowering striatal glutamate levels. In addition, 6-hydroxydopamine-lesioned R6/2 mice show extended survival along with a significant reduction in striatal glutamate.

### 2. *Defective energy metabolism toxin models*

While an increased endogenous excitotoxin or an abnormality affecting the NMDA receptor could be responsible for HD, candidate endogenous neurotoxins, however, are not increased in HD. One hypothesis explaining the pattern of degeneration in HD suggests that impaired cellular energy is involved in the degenerative process (Albin and Greenamyre, 1992; Beal, 1992). The initial and relevant observations showed that partial membrane depolarization produces NMDA receptor-mediated excitotoxicity by removing the voltage-dependent magnesium block of the NMDA-linked calcium channel (Novelli *et al.*, 1988;

Olney, 2011). The open calcium channel allows normal amounts of endogenous glutamate to induce NMDA receptor-mediated neurotoxicity. It has been reported that energy depletion producing partial membrane depolarization also results in NMDA-type excitotoxiclesions (Novelli *et al.*, 1988; Zeevalk and Nicklas, 1991). A proton gradient generated by the electron transport chain stores potential energy for the synthesis of ATP along with reducing oxygen to water. As such, energy failure may be the consequence of impaired electron transport chain function, with subsequent reduced ATP stores, resulting in membrane depolarization, removal of magnesium from the NMDA-linked calcium channel, and subsequent excitotoxic injury.

A number of electron transport chain enzymes that have been reported to be altered in HD. Studies of platelets from HD patients suggest that Complex I activity may also be selectively decreased in HD patients, although Complex l activity is normal in at-risk family members (Parker *et al.*, 1990). It is of interest to note that other electron transport chain complexes, including Complex II (succinate ubiquinol oxidoreductase), Complex III (ubiquinol cytochrome c reductase), and Complex IV (cytochromeoxidase) are normal in blood platelets (Parker *et al.*, 1990). Selective decreased activity of Complex II/III of the electron transport chain is present in the caudate nucleus, but not in other brain areas in HD (Mann *et al.*, 1990). While there is a reduction in Complex II–III activity in the caudate nucleus, there is a significant increase in Complex I activity in the frontal cortex, that latter of which may be compensatory (Browne and Beal, 1994). While cytochrome oxidase (Complex IV) abnormalities have been described in HD caudate nucleus (Brennan *et al.*, 1985), the loss of cytochrome oxidase in damaged brain areas may be a consequence of neuronal loss rather than a causal event. It is possible, however, that the gene mutation in HD affects a nuclear-encoded component of Complex II, Complex I, or the process of protein translocation into mitochondria.

Animal studies show that striatal injections of mitochondrial toxins produce differential neuronal toxicity identical to that produced by NMDA receptor agonists (Beal *et al.*, 1991c; Schulz *et al.*, 1995a; Storey *et al.*, 1992). Several specific inhibitors, such as malonateand 3-nitropropionic acid (3-NP), act at various complexes of the electron transport chain. A naturally occurring plant toxin and mycotoxin, 3-NP, is an irreversible inhibitor of succinate dehydrogenase that inhibits both the Krebs cycle and Complex II activity of the electron transport chain. Ingestion in cattle causesdyspnea, hindlimb weakness, and motor abnormalities (Alston *et al.*, 1977; Ludolph *et al.*, 1991). In China, accidental human systemic ingestion of 3-NP from contaminated sugarcane has resulted in neurological sequelae that include encephalopathy with stupor and subsequent coma, delayed-onsetnonprogressive dystonia with jerk-like movements, and facial grimacingin recovering patients. Brain imaging identified bilateral damage to the basal ganglia, particularly, the putamen. 3-NP reduces cellular levels of ATP and causes

neuronal damage by an excitotoxic mechanism (Ludolph *et al.*, 1992). Systemic 3-NP administration in rats and primates produce selective striatal lesions that are a consequence of secondary excitotoxic mechanisms (Beal *et al.*, 1993; Brouillet *et al.*, 1993). The selesions accurately replicate motor and neuropathological symptoms observed in HD patients, resulting in differential sparing of striatal NADPH-diaphorase and large cholinergicneurons with a significant loss of striatal GABAergic neurons, consistent with findings in HD patients. Bothenkephalin and substance P striatal neurons, however, are equally affected by 3NP, a finding that is inconsistent with those present inadult-onset HD (Sun *et al.*, 2002). 3-NP also shows an age-dependent neurotoxicity. Both freeze-clamp measurements and chemical shift magnetic resonance spectroscopy show that 3-NP impairs energy metabolism in the striatum *in vivo*. While intrastriatal injection of 3-NP results in striatal neuronal death in rats, neurochemical and histologic evaluation shows that markers of both spiny projection neurons (GABA, substance P, calbindin) and aspiny interneurons (somatostatin, neuropeptide Y, NADPH-diaphorase) are equally affected by intrastriatal injections (Beal *et al.*, 1993). Interestingly, the lesions produced by intrastriatal injection orsystemic administration of 3-NP are blocked by prior decortication, suggesting that intact corticostriatal glutamatergic innervation plays an important role in striatal degeneration. As indicated above, deafferentation of the substantia nigra and cortex is neuroprotective in transgenic HD mice (Stack *et al.*, 2007a). Since glutamate is a central tenant in provoking excitotoxic cell death in striatal neurons already weakened by the collective molecular events occurring in HD, it is of interest to note that the modulation of other neurotransmitters, such as dopamine, can contribute to the neurodegeneration in HD. In addition, the modulation of A2a receptors can ameliorate 3-NP-induced neuronaldamage (Blum *et al.*, 2002). In primates, chronic 3-NP administration produces selective bilateral striatal lesions characterized by a depletion of calbindin neurons with sparing of NADPH-diaphorase neurons, and proliferative changes in the dendrites of spiny neurons.

Lesion approaches in experimental animals using selective neurotoxins have made it possible to clarify the mechanisms that underlie the hyperkinesis (Crossman *et al.*, 1987, 1988; DeLong, 1990). It has been suggested that there is a shift in the pathways of the basal ganglia/thalamocortical circuit in HD (DeLong, 1990). Animals also show both spontaneous and apomorphine inducible choreiform movement disorders resembling those in HD. These findings clearly associate metabolic stress and striatal neuronvulnerability. The differences in the periodicity of excitotoxin injections and total dosing levels result in the reported differences in susceptibility. Acute treatments consisting of a single i.p. dose of 3-NP rapidly lead to striatal degeneration within 6–12 h after injection (Alexi *et al.*, 1998; Bizat *et al.*, 2003; Brouillet *et al.*, 1998). Subacute treatments consisting of daily repeated intraperitoneal injections lead to striatal degeneration over a few days (Beal *et al.*, 1993; Guyot *et al.*, 1997a; Schulz *et al.*, 1995b). The degree of

toxicity in rodents is also dependent upon the strain and gender of experimental animal used (Brouillet *et al.*, 2005). Systemic administration of 3-NP, however, is not specific to the CNS alone. There is now strong evidence to suggest that 3-NP has severe cardiotoxic effects in addition to neurotoxicity (Gabrielson *et al.*, 2001) and, as such, the underlying cause of the mortality and clinical phenomena observed in 3-NP toxicity are questioned. Peripheral cardiac damage may contribute to the clinical and neuropathological outcome measures from hypoxia. As such, postmortem analysis of somatic organs, especially the heart, in assessing pathological involvement is critical in determining the involvement of systemic and neurological contributions to CNS damage using 3-NP. While 3-NP toxicity replicates a number of the cell death mechanisms associated with HD, genetic mutant huntingtin rodent models may provide the best possible molecular and pathophysiological correlation to humans with HD. Although toxin models do not help to advance our understanding of the pathogenicity of expanded polyglutamine tracts, this is not to suggest that the 3-NP model is not without merit, as it continues to enrich our understanding of specific pathophysiological phenomena in HD associated with excitotoxicity and mitochondrial dysfunction.

### B. Genetic Models of HD

Genetic models have revolutionized the study of human neurological diseases by providing accurate and experimentally accessible systems in which to study molecular pathogenesis. Genetic models also provide an opportunity to test potential treatments and explore their promise for translation to humans experiencing these diseases. This is perhaps greatest in inherited diseases, such as HD, which affect single genes. It must be said, however, that even the genetic models of HD are not perfect since there are subtle differences in the huntingtin gene from the human orthologue along with dissimilar promoters. It has also been suggested that since the length of polyglutamine repeats in some genetic models represents the more fulminant juvenile form of HD with little resemblance to adult onset HD. The degree of overexpression of mutant protein plays a significant role in the phenotype observed in mice. The gold standard is the human condition and no genetic model replicates all of the findings in HD patients. Each model, however, has valid and useful experimental outcomes that can be used to provide a greater understanding of the disease process in humans and especially in identifying potential therapeutic strategies.

Different genetic mouse lines have been generated with varying phenotypes as a product of how the mutant huntingtin was incorporated into the mouse genome. They fall into three broad categories: (1) mice that express exon-1 or exon-1 and 2 of the human huntingtin (htt) gene containing polyglutamine mutations (in addition to both alleles of murine wild-type huntingtin, Hdh) (Jenkins *et al.*, 1999;

Laforet *et al.*, 2001; Mangiarini *et al.*, 1996); (2) mice with pathogenic CAG repeats inserted into the existing CAG expansion in murine Hdh (knock-in mice) (Heng *et al.*, 2007; Lin *et al.*, 2001; Menalled *et al.*, 2003; Shelbourne *et al.*, 1999; White *et al.*, 1997; Wheeler *et al.*, 1999, 2000); and (3) mice that express the full-length human HD gene (plus murine Hdh) (Hodgson *et al.*, 1996; Reddy *et al.*, 1998). Although all of these models share features with human HD, the phenotype of full-length huntingtin mutation models develops gradually over many months and may not have a sufficient expression of disease to use progressive morbidity and survival as endpoints. HD progresses over many years in patients and the exact expression of the clinical and pathological phenomena observed in patients may not be present in short-lived animal. While the full-length models are genetically more accurate, the fragment models have a rapidly coursing robust phenotype, well-defined neurobehavioral and neuropathological findings, and die between 3 and 5 months of age. It has been a common practice to use the fragment models for therapeutics research because the outcomes are more clearly established and trials are more easily conducted (Hersch and Ferrante, 2004). The ideal transgenic mouse model should have a moderate disease onset and progression, well-defined behavioral abnormalities, and neuropathological features, all of which accurately replicate human HD.

### 1. *Fragment/Segment Genetic Murine Models of Human HD*

In the fragment models of HD, affected mice express N-terminal fragments of human HD along with both alleles of murine Hdh. Not surprisingly, the degree of similarity to human HD increases the closer the model reproduces the exact neuropathological and molecular conditions for HD. However, as indicated above, the more genetically accurate the model is, disease phenotype is less fulminant and rapidly coursing. Thus, it has so far been much more feasible to use the fragment models for therapeutic research because the outcomes are more prevalent and definable without great variability in diagnostic criteria. This category of genetic mice expresses N-terminal fragments of human HD and includes the R6/2, R6/1, and N171-82Q lines. They show a relatively rapid onset and progression of a phenotype that includes weight loss, motor performance abnormalities, neuropathological sequelae, and a shortened lifespan.

### 2. *R6/2 Transgenic Mice*

The R6 line was developed in the laboratories of Gill Bates from a pronuclear injection of a 1.9 kb SacI-EcoRI fragment using the 5' end of the human HD gene derived from an HD patient. It is composed of ~1 kb of 5' UTR sequences, exon 1 carrying CAG repeats of ~130 units, and the first 262 bp of intron 1. The R6/2 line was the first transgenic mouse model of HD. It has a peak size of 144–150

repeat units at exon 1 (Mangiarini *et al.*, 1996). It is one of the most widely employed genetic models of HD. The R6/2 model exhibits a progressive homogeneous HD-like phenotype, with survival ranging from 14 to 21 weeks, depending on housing and facility conditions. Differences in survival duration may be the result of variations in housing, handling, environmental enrichment, and the allowable presence of viral and bacterial symbionts, in addition to other factors. Given that enriched environments can alter the progression of behavioral phenotype in R6/2 mice (Hannan, 2004), it stands to reason that differences between laboratories may well alter the R6/2 phenotype. R6/2 mice presently available in commercial vivaria have a much lower CAG repeat than those previously used in experimental studies. The above issues mandate that, within any colony of R6/2 mice, CAG repeat size is critical to any findings and a repeatable measurement of survival and other phenotypic outcome measures are paramount for each successive "f"generation for comparison to other studies.

Behavioral analyses of the R6/2 mouse showage-related impairments in dystonic movements, motor performance, grip strength, and body weight that progressively worsen until death. R6/2 mice are prone seizure activity (status epilepticus) and sudden death, particularly in end-stage disease, although seizures may occur as early as 60 days. Overhandling and other stressors exacerbate seizure activity. Neuropathological sequelae which include increasing marked reductions in brain weight, are present from 30 days, whereas decreased brain volume and hyperventricular enlargement is present from 60 days, both a hallmark of the human disease. In addition, decreased neostriatal volume, striatal neuron atrophy, increased astrogliosis, and a reduction in striatal neuron number, are present at 90 days of age (Stack *et al.*, 2005). In addition, consistent with early adult-onset HD, enkephalin striatal neurons are reduced in comparison to substance-P striatal projection neurons (Sun *et al.*, 2002) with equal preservation of enkephalin and substance-P striatonigral projections. Huntingtin-positive aggregates are present at postnatal day 1 and increase in number and size with age, suggesting that disease onset and progression occur before the presence of clinical phenomena (Stack *et al.*, 2005). The huntingtin inclusions are extensive and found throughout the brain in great numbers, a phenomenon that is inconsistent with that observed in HD patients. It has been suggested that the latter may be the result of using only a portion of the HD gene, transgene effects, and/or the use of foreign promoters that increase expression levels. There does not appear to be any gender differences in the pathological phenotype. There is parallelism between the reported mechanisms of disease pathogenesis observed in HD patients and those found in the R6/2 mice, which include altered proteolysis and proteosomal activities, increased protein crosslinking, induced chaperone expression, and defects in vital cellular processes that comprise endocytosis, intraneuronal trafficking, transcriptional regulation, postsynaptic signaling, apoptotic cascades, and alterations in bioenergetic metabolism and mitochondrial function (Beal and Ferrante, 2004; Ryu *et al.*, 2005; Stack

and Ferrante, 2007). While the R6/2 model has many of the clinical and neuropathological features observed in HD patients, it is not an exact genetic and neuropathological match with HD patients. Nevertheless, the R6/2 model has a well-characterized progressive phenotype with moderate variability, such that experimental groups can contain as few as 10 mice and provide the power to detect differences in many outcome measures. It is possible to perform survival studies, an important potential surrogate indicator for neuroprotection, in approximately 3 months from birth. The efficiency and clear experimental endpoints of the R6/2 mice remain a major advantage.

There can be great variability in phenotype presentation, which is dependent on CAG repeat size. The number of CAG repeats in the R6/2 line is 148–153 with 500–550 bp, as determined by PCR analysis (Stack *et al.*, 2005). An increased number of base pairs >550 results in a moderation of the R6/2 phenotype severity. With increasing base pair numbers, there is a concomitant increase in CAG repeat size. Base pairs ranging between 600 and 800 have CAG repeat sizes between 175 and 192 in R6/2 mice and the average survival extension significantly increases to approximately 131 days, in contrast to 500–550 bp at 98 days. Base pair numbers of 1000 and above have CAG repeat sizes consistently above 200, with a mean survival of 148 days. The variability in survival and the amelioration of the behavioral and neuropathological phenotype in R6/2 mice with increased base pair number and CAG repeat size may reduce their utility in therapeutic trials and may confound experimental results (Stack *et al.*, 2005). Although great variability in clinical measures is common in human trials, minimizing measurement variability increases the power to detect differences, particularly in mouse drug trials. Thus laboratories using these mice should ensure that genetic variability is reduced, providing a relatively homogeneous population of mice within experimental cohorts.

### 3. *R6/1 Transgenic Mice*

The R6/1 mice were developed along with the R6/2 mice and express exon 1 of the human HD gene with approximately 116 CAG repeats. The R6/1 mice have not been as well studied as the R6/2 mice (Mangiarini *et al.*, 1996). The R6/1 line has a later age of onset and a slower disease progression and may be the result of the reduced CAG repeat size. Body weight loss occurs after 22 weeks (Naver *et al.*, 2003). They exhibit motor performance abnormalities, as limb clasping, at 4–5 months. There is a significant decline in rotarod performance between 14 and 20 weeks. This behavioral change correlates with the presence of huntingtin aggregates in striatal neurons. Gait abnormalities and hindlimb clasping are similar to the R6/2 mice, although their life span is beyond 12 months. Brain volume is significantly reduced by 18 weeks with striatal neuron atrophy. There is, however, no neuronal loss, as identified by neuron-specific nuclear

protein NeuN or met-enkephalin immunostaining in the striatum (Naver *et al.*, 2003). Huntingtin aggregates are present by 2 months of age. While both R6/1 and R6/2 mice are resistant to excitotoxic lesions produced by quinolinic acid and malonate (Hansson *et al.*, 1999, 2001a), it is possible that the latter may be attributable to a reduction in dopamine levels in the R6/1 mice and R6/2 mice (Hickey *et al.*, 2002), (Petersen *et al.*, 2002a). In the R6/1 mice, extracellular dopamine levels are reduced by 70% and intrastriatal administration of malonate in these mice results in significantly smaller lesion size (Petersen *et al.*, 2002b). In contrast, R6/2 mice are more susceptible to the mitochondrial toxin 3-NP (Bogdanov *et al.*, 1998). It may well be that differences in the periodicity of excitotoxin injections and in total dosing levels between reporting laboratories result in the published differences in susceptibility of 3-NP. Equally, background strain may also play a role in resistance (McLin *et al.*, 2006). In addition, reduced sensitivity to excitotoxins may be dependent upon age and CAG repeat length (Hansson *et al.*, 2001b).

4. *N171-82Q Transgenic HD Mice*

N171-82Q mice, developed by David Borchelt, have an N-terminal fragment of huntingtin incorporating both exon 1 and exon 2 of the huntingtin gene, with 82 polyglutamines (Jenkins *et al.*, 1999). The N-terminal fragment is driven by the mouse prion promoter and expression is restricted to neurons and no other cells in the CNS. The mice contain two wild-type copies of the gene along with one mutant copy. The phenotype of N171-82Q mice is similar to, but less severe than that present in the R6/2 mice. The first abnormality observed is weight-gain failure with a significant body weight loss over the last 6 weeks of life. In contrast to the R6 lines of HD mice, seizure activity and hyperkinesis are not present in the N171-82Q mice. They do, however, show deficits in the radial arm water maze test of working and reference memory at 14 weeks (Ramaswamy *et al.*, in press). The life span is variable, ranging from 130 to180 days. The neuropathological features of the N171-82Q mouse models are similar to the R6 mice in that they show striatal atrophy, hyperventricular enlargement, striatal neuron loss, and astrogliosis (McBride *et al.*, 2006; Yu *et al.*, 2003). Huntingtin and ubiquitin-positive inclusions are found as early as 16 weeks and continue to increase in number through end-stage disease, particularly in the pyriform cortex. The phenotype of N171-82Q mice, however, is more variable than that of R6/2 mice and, as such, a greater number of N171-82Q mice ($n = 20$) are necessary for experimental trials.

5. *Murine Hungtintin Homolog Knock-In Mice*

While this group of HD mice represents a more precise genetic model of HD, the knock-in mice models present with a mild and protracted behavioral and

neuropathological phenotype with a normal lifespan, in contrast to the fragment models. An expanded CAG repeat is inserted into the murine huntingtin homolog and, therefore, the mutation is genomically correct and under the endogenous Hdh promoter. The mice can be homozygous or heterozygous for the mutation. While multiple knock-in models have been created, four are most widely used and include the HdhQ111, the Hdh/Q72-80, the CAG140, and the CAG150 HD mice. The chimeric HdhQ lines were developed by a mutated exon 1 containing either 111 or 92 CAG repeats. These large polyglutamine repeats cause a CAG repeat instability, predisposing them to subsequent increases in CAG repeat, resulting in a more fulminant disease process (Wheeler *et al.*, 1999). Huntingtin-positive puncta are present in neuronal nuclei by 4 and a half months along with an increase in gliosis by 24 months in both lines (Wheeler *et al.*, 2000). Mitochondria undergo compensatory changes in calcium sensitivity in both the Q92 and Q111 mice (Brustovetsky *et al.*, 2005). A knock-in Hdh mouse with 72–80 CAG repeats shows aggressive behavior, rotarod impairment, and neuropil aggregates, but no gliosis or neuronal loss (Shelbourne *et al.*, 1999).

The HdhQ111 knock-in mice have 111 CAG repeats inserted into the murine HD gene (Wheeler *et al.*, 2000) and develop a progressive neuropathological phenotype, consisting of nuclear localization of the full-length huntingtin protein in medium striatal spiny neurons, and subsequent formation of N-terminal inclusions and insoluble aggregates. They develop a late-onset neurodegeneration and gait deficit at 24 months of age (Wheeler *et al.*, 2002). While no differences in rotarod abnormalities are present, paw-clasping, tunnel walk, and stride length outcome measures reveal a 'subtle' gait deficit. Reactive gliosis and toluidine-blue stained striatal neurons were present at 24 months. These neurons are negative for TUNNEL staining, again suggesting a very "subtle" disease progression as reported by the authors.

A knock-in mouse with 94 CAG repeats has increased rearing at night at 2 months of age, decreased activity at 4 and 6 months, but a normal lifespan. Nuclear microaggregates are present at 4 months and are widely distributed at 6 months of age in these mice. In mice developed by the same group with 140 CAG repeats, a similar pattern of motor abnormalities is present (Menalled *et al.*, 2003). Disease onset in the 140 CAG mice occurs much earlier than in the 94 CAG mice, consistent with the same phenomena in HD patients, as the onset of symptoms in HD patients occurs at a younger age with greater CAG repeat size. In addition, there is increased motor activity and rearing at 1 month of age, hypoactivity at 4 months of age, and a reduction in stride length at 12 months of age. No significant weight loss is reported up to 1 year of age. Further analysis, however, has shown a much wider range of motor and behavioral dysfunction (Hickey *et al.*, 2008). Open field analyses in 140 CAG mice of distance traveled, ambulatory counts, resting time, and ambulatory time showed significant differences from only the 3-month time point onward, in comparison to littermate control mice, suggesting that the

2-month time point may be a clinically premanifest time point (Kim *et al.*, 2010b). While neuronal loss and reactive astrogliosis are not observed in the 94 CAG, they are present in the 140 CAG mice. The 140 CAG mice show decreases in dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP32) (a marker of striatal projection neurons) at 12 months, and neuronal loss at 2 years of age (Hickey *et al.*, 2008). Huntingtin aggregates are present in both the 94 CAG and 140 CAG mice. With age, there is a more global presence of huntingtin aggregates throughout the CNS. There is a derangement in bioenergetic mechanisms, increased oxidative stress, and factors associated with transcription regulation in the 140 CAG mice, with reduced ATP brain levels, increased 8-hydroxy 2'-deoxyguanosine (a marker of DNA oxidation in both brain and urine), hypoacetylation of histone activities (Foran *et al.*, 2006), and a reduction in creatine kinase activity in blood and brain (Kim *et al.*, 2010b). The 140 CAG mice may be useful in premanifest and symptomatic HD investigations, as well as for therapeutic evaluation.

The 150 CAG repeat knock-in mouse model has late-onset gait abnormalities and develops neuronal intranuclear inclusions predominantly in the striatum (Heng *et al.*, 2007; Lin *et al.*, 2001; Tallaksen-Greene *et al.*, 2005). There is an age-dependent late-onset behavioral phenotype with significant motor abnormalities at 70 and 100 weeks of age measured by rotarod, balance beam, and clasping (Heng *et al.*, 2007). At 100 weeks, the mice exhibit resting tremor, unsteady movements, and staggering gait. No significant gender differences have been observed. There is significant weight loss by 70 weeks with continued weight reduction with age. Gliosis is significantly increased by 14 months. Further analysis of this model shows that striatal nuclear huntingtin and ubiquitin-positive inclusions are associated with the matrix compartment by 27 weeks, the topographic striatal area first thought to be involved in HD patients (Guyot *et al.*, 1997a). In older animals, nuclear inclusions are distributed evenly in both the striatal patch and matrix compartments by ~2 years, nuclear inclusions are present in most brain areas. Measurements of dopamine D1 and D2 receptor binding sites are reduced by at 70- and 100-week time points. Striatal neuron loss is present at 100 weeks, with a 50% loss in striatal perikarya and a 40% reduction in striatal volume. Recent evidence shows NMDA receptor-mediated excitotoxicity in this mouse line, providing additional proof that the interaction of huntingtin with NMDA receptors may be an early event in neuronal death in HD (Heng *et al.*, 2007). Further longitudinal analysis of the behavioral phenotype in these mice shows early and progressive cognitive deficits along with impaired motor performance (Brooks *et al.*, 2010). As with the 140 CAG HD mice, the Hdh-150 mice may be useful in identifying early disease mechanisms and premanifest biomarkers of HD.

While the knock-in mouse lines do not have a rapid and fulminant disease progression to use early morbidity and survival as outcome benchmarks, they do have a number of measurable neuropathological and behavioral phenotypes that can be validated as potential endpoints in therapeutic studies and may be useful as

both a primary model, as well as a secondary model for confirmation. A greater investigation into the pathophysiological phenomena of these mice is ongoing.

### 6. *Full-length Human HD Gene Transgenic Mouse Models*

Transgenic mice with full-length huntingtin containing 48 or 89 CAG repeats have been described and are reported to have behavioral abnormalities along with neuronal loss (Reddy *et al.*, 1998). A yeast artificial chromosome (YAC) mouse model of HD with the full human huntingtin gene and containing 128 CAG repeats (YAC 128) develops motor abnormalities consisting of hyperactivity followed by abnormalities in walking from 6 to 12 months, with subsequent hypokinesis (Hodgson *et al.*, 1999; Slow *et al.*, 2003). There are other significant declines in motor performance starting as early as 3 months and include circling behavior, hindlimb clasping, and gait abnormalities. In addition, rotarod and open field abnormalities are present at 4 and 2 months, respectively (Van Raamsdonk *et al.*, 2007). While there is marked body weight loss in the YAC72 mice, there is an increase in body weight in the YAC128 mouse by 2 months (Van Raamsdonk *et al.*, 2007). Cognitive dysfunction, measured by the swim test for procedural learning, has been compared to the perseveration observed in HD patients. The motor deficit in the YAC128 mice correlates with striatal and cortical neuron loss, providing a structural correlate for the behavioral changes observed in these mice (Slow *et al.*, 2003). Neuropathological examination shows the presence of huntingtin immunostaining at 1–2 months, huntingtin macroggregates starting at 12 months and increasing in number by 18 months, with decreased striatal and cortical volume and reduced striatal neuron area and number by 12 months in the YAC128 model (Van Raamsdonk *et al.*, 2007). The YAC 128 model has been used extensively to investigate pathogenic mechanisms of HD and most recently to identify therapeutic strategies. Medium-sized spiny striatal neurons are more vulnerable to NMDA receptor-mediated death in the YAC transgenic mouse model of HD expressing full-length mutant huntingtin (Zeron *et al.*, 2002). Both mitochondrial and apoptotic pathways are altered in these mice (Fernandes *et al.*, 2007). The neuropathology in the YAC mice has excellent fidelity with human HD. Recent studies have shown poly Q length-dependent enhancement of peak NMDA receptor current density and NMDA receptor trafficking, along with enhanced NMDA-induced apoptosis (Fan *et al.*, 2007; Fernandes *et al.*, 2007).

Transgenic mice using a bacterial artificial chromosome (BAC) look promising (Gray *et al.*, 2008). These mice have a 240 kb BAC that contains the entire 170 kb human huntingtin locus. These mice show progressive motor deficits, exhibiting a significant reduction in rotarod performance at 2 months with worsening behavior through 12 months. The BACHD mice also show a significant gain in body weight, similar to the YAC 128 mice, that plateaus at 12 months. By 12 months, there is marked gross brain atrophy and brain weight loss, with significant

decreases in both cortical and striatal volume. Degenerating dark neurons were present in the striatum at 12 months without significant neuronal loss. Huntingtin inclusions were detected starting at 12 months and are present almost entirely within the cortex withsome inclusions in the stritum. Electrophysiological studies show that spontaneous excitatory transmission is significantly reduced in the BACHD mice, suggesting early abnormalities in cortical input to striatal neurons. Although these mice have not yet been used in a published therapeutic discovery experiment, there are efforts underway.

A HD transgenic rat model has been developed that expresses 51 human CAG repeats (von Horsten *et al.*, 2003). Expression of the gene is under an endogenous rat promoter. While normal at birth, there is a progressive reduced performance on rotarod after 2 months, gait abnormalities and head dyskinesias starting at 10 months, cognitive deficits by 12 months, and significant body weight loss by 24 months (Nguyen *et al.*, 2006).The neuropathological sequelae include hyperventricular enlargement, along with huntingtin inclusions throughout the brain.

### C. Lentiviral-Mediated Mutant Huntingtin Model

A lentiviral delivery of mutant huntingtin has been developed in a rat model using 44, 66, and 82 repeat fragments (de Almeida *et al.*, 2002). There is increased expression of ubiquitinated htt aggregates starting 1 week after injection with great numbers of inclusions by 4 weeks. Striatal neuron degeneration, loss of DARPP-32 staining, and cell death occur by 6 months. The neurodegeneration is specific to spinal striatal GABAergic neurons, with little or change in striatal interneurons. These findings are consistent with observations in HD patients.

### D. Non-Human Primate Models of HD

While 3-NP toxin lesions have been used as an experimental model of HD in primates and closely parallel the neuropathological, neurochemical, and clinical features of HD, a transgenic HD rhesus monkey model has been developed that expresses expanded huntingtin polyglutamine. These primates show clinical symptoms that include dystonia and chorea similar to the clinical phenomena observed in HD patients (Yang *et al.*, 2008). Although huntingtin aggregates are present in the brains of these monkeys, no striatal neuron degeneration has been found. As HD is a slowly progressive disorder, it may be years before these monkeys show a full HD neuropathological phenotype. Clinical follow-up continues with surviving monkeys. The availability of nonhuman primates with HD could be valuable in any final analysis evaluating the most promising therapeutic candidates for HD patients.

## III. Methodological Considerations for Mouse Therapeutic Trials

While the design of this chapter was not meant to review in detail the methodologies used in preclinical trials to help determine potential translation of therapies to patients with HD, it is worthwhile to mention the importance of the basic guidelines that are vital to ensure that animal trials are reproducible and valid. Although making the therapeutic leap from genetic models to humans fulfills one of the promises of molecular medicine, it also brings risk to human subjects and expends human and financial resources. Thus, how data from experiments using experimental models are used to inform choices about clinical trials requires reflection and examination. Among the important issues are what constitutes an informative genetic model, what principals should be followed in designing experiments using these models, what body of evidence is desirable to fully inform clinical decision making, and what factors contribute to the equipoise in determining whether preclinical information about a therapy makes human study warranted.

There is a growing body of evidence suggesting that the phenotypes from mouse models of neurological diseases closely correlate with human diseases and may validate known CNS drug targets in a therapeutically relevant manner. The strengths of the HD mouse models are in their utility to provide parallel pathophysiological targets that are present in HD patients, in their potential as sensitive predictors for therapeutic intervention, and their promise in the development of novel drug agents. While drug trials in mice confirm therapeutic direction, the challenge is in determining what dose might be of value in patients since the pharmacokinetics of mice and man is dissimilar. The lack of any proven neuroprotective therapy for HD also affects the equation by keeping the level of urgency high. Unfortunately, it has not yet been confirmed that experiments demonstrating improved phenotype in HD mice are predictive of benefits in humans. There have been, however, recent studies showing significant parallels in biomarkers of disease pathogenesis and treatment efficacy from both HD patients and HD mice (Foran *et al.*, 2006; Hersch *et al.*, 2006; Kim *et al.*, 2010b). Similarly, it is unknown whether the magnitude of benefit in mice predicts the magnitude of benefit in humans. At the moment, the findings using bioenergetics compounds, such as creatine and coenzyme $Q_{10}$, in HD patients and HD mice have provided intriguing information that lends promise to this issue. There is every belief that the current high-dose creatine trial in HD subjects will bring to light the value of mouse clinical trials.

While toxin models play a role in understanding mechanisms of excitotoxicity and mitochondrial dysfunction in HD, they fall short as a reliable model of an autosomal dominant genetic disease characterized by the misfolding of the mutant huntingtin protein. Genetic animal models of inherited neurological diseases provide an opportunity to test potential treatments and explore their promise

for translation to humans experiencing these diseases. Therapeutic trials conducted in genetic mouse models of HD have identified a growing number of potential therapies that are candidates for clinical trials and have been reviewed in multiple publication venues (Beal and Ferrante, 2004; Stack and Ferrante, 2007). There is a need, however, to begin to prioritize the many leads emerging from transgenic mouse studies. This can only happen if there is some standardization of experimental methodologies and outcome measures in transgenic mouse models of HD. To date, it has been very difficult to compare results between compounds and laboratories and there are also many additional factors that can affect translation to humans. As such, the use of appropriate methodology in human clinical trials and in mouse trials to determine outcomes should have the same considerations apply to both, but are not always considered in mouse trials. While there are many different outcomes that could be used in characterizing drug efficacy, the following minimum guideline criteria are essential to strengthen the evidence for drug translation to patients.

Pharmacokinetic analysis of the study compound, its metabolites, or of molecules expected to be affected by the treatment, help to insure that the compound reaches the brain and performs as expected. Additionally, understanding effective doses in mice, along with blood and brain levels, can set the stage for asking whether such doses and levels can be achieved in humans. HPLC with electrochemical detection along with spectrometric analysis are the methods of choice (Dedeoglu *et al.*, 2002, 2003). Because pharmacokinetic studies provide critical information regarding optimal dosing, maximum-tolerated-dose studies are important to determine the LD50 dose starting at a minimum tolerated dose and increasing the dose onefold b.i.d. each administration over the course of 14 days or until LD50 is reached. Latency as a time-concentration curve in blood and brain provides information regarding dose frequency. These experimental studies are completed in both wild-type and mutant mice prior to a drug trial in mice and allow a determination of the optimal pharmacokinetic parameters for dosing the mice. In addition to pharmacokinetic studies, it can be very helpful to perform pharmacodynamic studies aimed at determining whether a compound's expected mechanism of action is actually working.

Blinding of study personnel about the treatment condition of the animals they are studying should be standard practice. A *priori* power analysis is essential to establish whether the study has sufficient numbers of mice for each endpoint. An estimation of the sample size is a critical first step. The numbers of mice for preclinical studies must be carefully considered and reflect the different outcome measures to be analyzed. Whereas in human trials enormous variability is a given, in mouse trials it is possible to minimize the variability and thereby increase the power to detect smaller differences in smaller groups. It is important to understand the genetic, physical, and environmental sources of variability to take advantage of this.

As in human clinical research, it is important to have inclusion and exclusion criteria, which should be predetermined. For example, there is good reason to consider excluding "runts" by weight, mice with injuries, mice in which CAG repeat length is out of the median range. CAG repeat must be monitored in the experimental colony within the laboratory vivarium or verified through the source of purchase. Treatment groups should be physically and genetically comparable. There should be an assignment of mice into experimental cohorts to prevent over-representation of sibs within any group. The environment in which these mice are housed and treated also needs to be uniform since environmental enrichment slows disease progression in HD mice and may be considered a therapeutic treatment in and of itself (Hockly *et al.*, 2002). Another important consideration is the onset and duration of treatment in mouse trials. Initiation of treatment after weaning, as has been most common, is analogous to treating presymptomatic patients. Beginning treatment once a clinical phenotype, such as motor deficits, is present would be analogous to most current human trials for HD.

There are many potential outcome measures that can be used in mouse therapeutic trials. These bear close examination because they can differ enormously in relevance with some clearly being much more informative and specific than others. For clarity, in neurodegenerative disease research neuroprotection is of the greatest importance, which at its most basic level is the preservation of neuronal processes, somata, and function. Measures that assess these directly (brain weight, gross atrophy, cellular atrophy, neuronal counts, gliosis, and volumetric imaging) should be considered the primary outcome measures. While it may also be possible to model the treatment of clinical symptoms in genetic models and it may also happen that improving behavioral symptoms corresponds to neuroprotection, assessments of clinical symptoms should be considered secondary outcome measures. These include survival, body weight, performance on motor and cognitive tasks, and laboratory studies examining toxicity or putative mechanisms of action. Clinical symptoms can be modified without affecting neurodegeneration. This distinction is important to keep in mind when considering how informative the results of a mouse therapeutic trial might be for translation to humans. Of those secondary measures that have been most useful, survival, or extension of life, is the most meaningful. Survival, or a surrogate for mouse survival, which are usually criteria for advanced morbidity that agrees with an institution's animal care policies and triggers euthanasia, is an especially useful secondary outcome measure. Besides generally correlating well with neuropathology, it provides a relevant measure of the magnitude of benefit that is both understandable and enables ready comparison with other therapies. A dose versus survival study is recommended, using at least three doses, to decide whether therapeutic benefits stabilize (saturate) or decline past the optimum dose determined by pharmacokinetic studies. In addition, laboratories should use positive control compounds to help place their results in context. For example, several labs

have replicated benefits with cystamine, so including a cystamine treatment cohort in trials of more novel therapeutics could help calibrate results (Stack and Ferrante, 2007). A treatment cannot be considered to be neuroprotective in mice in the absence of neuropathological evidence that brain atrophy, cellular atrophy, or neuronal loss has been prevented. The quality of neuropathology in mouse therapeutic trials is thus of foremost importance. Standardization of tissue processing is critical to the analysis. And quantitative methods are essential as observation alone can only detect large differences and semi-quantitative methods are prone to many types of errors. It should also be mentioned that other neuropathological measures, such as protein aggregate load or the expression of molecules of interest, are only meaningful if brain atrophy, cellular atrophy, or neuronal loss are measured to provide a context for interpreting them. Lastly, the use of both fragment and full-length HD mouse models in preclinical drug trials and confirmation of the experimental findings in at least one independent laboratory that is familiar with animal trials are important once the potential of a compound has been established.

### A. Biomarkers of HD

A major goal of current clinical research in HD is to improve early detection of disease and premanifest detection of neuronal dysfunction with translation to therapeutic trials. Biomarkers are urgently needed for diagnosis, disease progression, and for potential disease-modifying therapies that are being developed and evaluated in clinical trials, especially at the preclinical stage. The development of early premanifest biomarkers is of great importance, as these may improve the power and cost-effectiveness of drug trials. As the mechanism of pathogenesis in HD is not yet clear, biomarkers will be required at all stages of disease and will necessitate multiple combinations of detection methods. One complementary research strategy has been to perform parallel correlative biomarker studies in HD patients and animal models of HD and make comparisons. This approach takes advantage of advances being made in animal models to best understand the most effective therapeutic strategies. While many different approaches have been undertaken to identify biomarkers, profiling objective biomarker measurements of HD has proven somewhat difficult at the present time. The optimal biomarker would be easily measured, repeatable and reliable, be present at all stages of disease, progress linearly with disease, and correlate between mouse and man. The ideal biomarker would reflect, in a peripheral body fluid or tissue sample, disease in the central nervous system. The most useful biomarkers measure a primary outcome of the disease; however, secondary outcomes also reflect changes and can therefore be of use.

Neuroimaging provides a noninvasive, reproducible, and reliable measure with which to track the progression of HD, however, changes in structure tend to progress more slowly and can thus limit the usefulness of neuroimaging as a

biomarker. Imaging techniques such as MRI, fMRI, PET, and DTI have all been used to study changes in structure and connectivity in the HD brain. Such imaging has revealed changes in the presymptomatic and early-onset HD brain. There is selective white matter abnormalities in premanifest HD and are correlated with cognitive and clinical manifestations using DTI (Rosas *et al.*, 2006). MRI morphometry has been used as a potential biomarker of disease progression, showing selective topographical associations of cortical thinning with clinical features of HD (Rosas *et al.*, 2008) TRACK-HD, an ongoing multinational, observational study of HD designed to study longitudinal changes in the premanifest brain, has found that there is significant whole-brain atrophy and caudate atrophy in premanifest and early manifest disease brains as compared to controls over the course of a year. They have also found changes in cortical and subcortical gray and white matter atrophy over the course of a year (Tabrizi *et al.*, 2010). Cortical thinning has also shown promise as a biomarker. Cortical thinning has been found to be present in premanifest brains, showing changes over the course of a year (Rosas *et al.*, 2002), and these findings correlate with disease stage (Rosas *et al.*, 2008) and cognitive test results. Based on these promising results, a 3-year study involving more than 600 patients has begun to confirm these results.

It has also been shown that biomarkers of protein crosslinking are significantly elevated in both HD patients and in HD mice. Both transglutaminase and gamma glutamyl lysine activities are significantly increased in brain samples in a disease-dependent manner (Dedeoglu *et al.*, 2002). There is evidence that biomarkers of nucleosomal and bioenergetic dysfunction are present in both HD mice and HD patients, representing biomarkers as predictors of disease onset and progression (Stack and Ferrante, 2007). In addition, 8-hydroxy-2-deoxyguanosine, a marker of DNA oxidation, is significantly increased in brain and urine from HD patients and HD mice (Foran *et al.*, 2006; Hersch *et al.*, 2006). Of great interest are studies using high-dose creatine in HD patients and in HD mice, showing parallelism in biomarkers of disease pathogenesis and treatment efficacy. Creatine treatment significantly improves brain creatine levels and reduces urine levels of 8-hydroxy-2-deoxyguanosine, a marker of DNA oxidation, in both HD patients and mice (Foran *et al.*, 2006; Hersch *et al.*, 2006). While it has not yet been confirmed that therapeutic experiments demonstrating improved phenotype in HD mice are predictive of benefits in humans, the above evidence demonstrates that mice do provide value.

Recent studies have shown that the brainisoenzyme of creatine kinase, an enzyme important in buffering energy stores, was significantly reduced in presymptomatic and manifest disease in brain and blood buffy coat specimens in HD mice and HD patients (Kim *et al.*, 2010b). Correlative biomarker analysis in both mice and man will provide a more rapid and desirable body of evidence to fully inform clinical decision making. While the efficiency and clear experimental endpoints of the R6/2 mice remain a major advantage, the fulminant and early

expression of disease in these mice may not allow for premanifest and early symptomatic identification of biomarkers. Full-length knock-in HD mice, however, may provide the best possible molecular and genetic comparability to human HD and strengthen the ability to confirm the detection of biomarkers, especially prior to disease onset, given their slow disease progression.

## IV. Existing Clinical Management

Existing medical care primarily focuses on symptom management, optimizing functions that are in continual decline, and the provision of ever-increasing levels of assistance. Symptomatic therapies using existing medications can help specific symptoms, such as depression, emotional dyscontrol, obsessive thinking, psychosis, chorea, rigidity, dysphagia, and weight loss. Nonpharmacological therapeutics are also very important and include occupational therapy, physical therapy, speech pathology, clinical nutrition, social services, genetic counseling, psychotherapy, and long-term care. All of these different medical, counseling, and rehabilitative modalities can ameliorate symptoms and help make HD more manageable for patients and their families. Although optimal care significantly improves the quality of life for HD patients, there is no evidence that it appreciably slows the progression of the disease.

Antipsychotic agents have been used to manage the depression, anxiety, and other psychiatric disturbances associated with HD. Selective serotonin uptake inhibitors such as sertraline and fluoxetine along with benzodiazepines such as clonazepam, diazepam, risperidone, and sulpiride have all been employed in the clinical setting (Ramaswamy *et al.*, 2007). However, no study has been conducted that provides significant evidence that any antipsychotic drug is more effective in the treatment of HD symptoms than any other. The use of other anxiotylics, such as Zyprexa and Seroquel, are gaining favor in HD. Both of these drugs have a secondary effect in improving weight gain, which is important in HD patients.

Although multiple drug agents have been employed to treat chorea, the dopamine inhibitor tetrabenazine is the most promising drug available and the only drug to be approved by the FDA for HD and was championed by Dr. Nancy Wexler (Fasano *et al.*, 2008; Group, 2006). Tetrabenazine works by reversibly inhibiting the central vesicular monoamine transporter type 2 (VMAT2) that causes dopamine depletion without greatly affecting norepinephrine levels. The site and action of binding makes tetrabenazine more useful than other dopamine depleting drugs for the treatment of chorea. Other dopamine depleting drugs such as reserpine bind VMAT1 in addition to VMAT2 and as VMAT1 is also present in the peripheral nervous system, reserpine causes adverse effects, such as orthostatic

hypotension. Tetrabenazine has the highest binding density in the areas most affected by HD, including the nucleus acumbens, the putamen, and the caudate nucleus. Because it binds reversibly, the monoamine depletion lasts only hours and is therefore not modified by long-term treatment. Adverse side effects include drowsiness, insomnia, depressed mood, agitation, akathisia, and hyperkinesia, but these effects disappear once patients reach the maximum dose. In addition to dopamine depleting agents, dopamine agonists, glutamate antagonists, and antiseizure medications are just a few of the other types of therapies that have been employed to treat chorea.

### A. Therapeutic Strategies

Many potential therapies have now been tested in genetic models of HD and some have been demonstrated to be neuroprotective in HD transgenic mice. Efficacy in mouse trials has provided the rationale for a number of clinical trials that have already occurred or are planned. These include coenzyme $Q_{10}$, creatine, remacemide, riluzole, minocycline, ethyl-epa, phenylbutyrate, and cysteamine (Stack and Ferrante, 2007). Most of these have been early-phase clinical trials, so the predictive value of mouse therapeutic trials are not yet known. However, because these are actually very good models at many levels of analysis, it would be surprising if some compounds benefiting the mouse models did not prove to benefit humans with HD. Since clinical trials may be expensive, effortful, and years in length depending on the type, there is growing discussion within neurodegenerative disorder clinical trials organizations, such as the Huntington Study Group and sponsors, about how much preclinical information is enough and how to prioritize among many efficacious compounds. Of course, the bar should be higher for later-phase trials and higher when the interventions have greater potential for risks to human subjects. The impact therapeutic trials in genetic models can have on selecting compounds for clinical trials in humans depends on many factors relating to the quality and breadth of the preclinical data, as well as on the potential risks and benefits to performing the human clinical trials.

## V. Mechanisms of Cell Death and Potential Therapeutic Targets in HD

### A. Mutant Huntingtin Aggregation (mHtt)

While the gene responsible for HD was discovered 18 years ago (A, 2011), the relationship between mHtt and the multiple molecular pathways that appear to

mediate neuronal death in HD remain to be clarified. There is a dynamic expansion of polyglutamine proteins from monomers to oligomers, with the latter thought to be pathogenic structures. These oligomers exist in both soluble form and as mHtt aggregates. These aggregates contain amyloid fibers and amorphous aggregates along with other proteins involved in the ubiquitin-proteasome and molecular chaperone system. Both the soluble and aggregate form have been suggested to cause neuronal dysfunction and cell death. While transglutaminase activity has been proposed to mediate mHtt aggregation (Zainelli *et al.*, 2005), there is ample evidence regarding transglutaminase expression in HD (Hoffner *et al.*, 2002; Karpuj *et al.*, 1999; Lesort *et al.*, 1999) and a role for transglutaminase in HD pathogenesis is now well accepted.

Interestingly, proteins containing polyglutamine expansions, such as mHtt, are degraded in a limited context by the ubiquitin-proteosome system (UPS) (Holmberg *et al.*, 2004). However, recent data suggests that the proteosome may not cleave polyglutamine sequences within the mutant protein (Holmberg *et al.*, 2004). Ongoing debate continues, as with other neurodegenerative disorders in which protein aggregates are a hallmark of disease, questioning whether inclusions formed by the aggregated N-terminal truncation of mHtt cause neuronal death through alterations of nuclear transport or DNA arrangements affecting transcription. Recent studies have suggested a protective role for aggregation (Arrasate *et al.*, 2004; Kuemmerle *et al.*, 1999; Taylor *et al.*, 2003). Through the use of an automated microscopy technique to assess the time frame in which neurons expressing mHtt expire, improved survival is seen in neurons that contain mHtt aggregates (Arrasate *et al.*, 2004). It is difficult, however, to reason that such factors as the mass effect of cytosolic and nuclear huntingtin aggregate burden, the sequestration of critical transcription factors and neuronal proteins that are essential for neuronal survival by huntingtin aggregates and their subsequent reduced activity, altered proteosomal function, and the localization of mutant huntingtin aggregates to cellular organelles such as mitochondria, do not have a deleterious effect on neuronal function and cell survival. One partial explanation put forth, however, is that a number of these deleterious effects may be caused by the dysregulation of the ubiquitin-proteasome dependent protein degradation pathway in response to polyQ-expanded huntingtin (Bence *et al.*, 2001). In defense of the latter, it has been reported that primary cortical neurons expressing full-length mutant Htt physically impede mitochondrial transport and that this is an early pathological event (Chang *et al.*, 2006). Immobilized mitochondria may result in reducing energy needs throughout the neuron, especially in striatal projection neurons. It is intriguing to suggest that the improved behavioral and neuropathological phenotype in HD mouse models by the administration of select therapies that reduce Htt aggregates (Beal and Ferrante, 2004) may be the result of, at least in part, improved mitochondrial dynamics and trafficking. One issue that remains unclear is the relation of mHtt aggregation to selective neuronal degeneration. In HD, a select

population of striatal neurons is affected while others are spared. As mutant huntingtin is present throughout the CNS, the hypothesis that mHtt causes cell death is insufficient to explain the selective degeneration. One possible explanation is that if cell death is dependent on the amount of soluble protein present in the cell, cells with higher levels of unaggregated mHtt will be more vulnerable (Arrasate *et al.*, 2004; Sisodia, 1998). It has also been suggested that amino acid residues outside of the expanded polyglutamines are responsible for selective toxicity (Gatchel and Zoghbi, 2005). Whether the formation of mHtt aggregates is protective or toxic, it is clear that coincident with mHtt aggregation, there are additional pathogenic cascades at work in HD.

As such, the inhibition and degradation of mHtt is a potential therapeutic target in HD. In normal neurons, organelle and protein turnover is a critical feature that promotes health and function. Altered proteolysis may result in aberrant protein changes in denaturation or misfolding. Two distinct routes mediating proteolysis in neurons are the ubiquitin-proteosomal pathway and the lysosomal pathway. Proteins destined for degradation mediated by the UPS must be tagged for degradation. In general, the UPS is responsible for the degradation of transiently expressed proteins (Ciechanover, 2006) and must be sufficiently unfolded to fit through the narrow opening of the proteosome (Pickart and VanDemark, 2000). In addition, degradation of proteins and organelles in bulk is accomplished through the lysosomal pathway, in a process termed autophagy (Rubinsztein, 2006). Through this pathway, cellular components destined for degradation are enveloped in double membrane bound vesicles, called autophagosomes, which fuse with lysosomes. Once fused, hydrolytic lysosomal enzymes degrade the contents. While the mechanisms regulating autophagy are not completely characterized, it is a process regulated by protein kinases, including the well-characterized mammalian target of rapamycin (mTOR) (Schmelzle and Hall, 2000). Phosphorylated mTOR is linked with protein synthesis, whereas dephosphorylation of mTOR induces autophagy (Somwar *et al.*, 1998). Furthermore, mTOR-mediated autophagy has been linked to glucose levels, with increased glucose stimulating autophagy and enhanced mHtt clearance through reduced mTOR phosphorylation (Ravikumar *et al.*, 2003). It is worth noting that autophagy can be induced through activity of the insulin receptor substrate-2, independent of mTOR activity (Yamamoto *et al.*, 2006). This results in a significant reduction of mHtt aggregation *in vitro* and is dependent on normal autophagosome formation mediated by Beclin1 and hVps34.

Notwithstanding, with the importance of mTOR in autophagy and the role of autophagy in HD, compounds that can interact with mTOR to promote autophagy may prove exceptionally beneficial in HD. The chemical induction of autophagy in reducing mutant aggregate proteins has been supported by the work of David Rubinsztein (Sarkar and Rubinsztein, 2008). Using specific inhibitors of autophagy 3-methyladenine or N6, N6-dimethyladenosine, the number and size

of mHtt aggregates increases (Ravikumar *et al.*, 2002). In contrast, induction of autophagy by rapamycin resulted in a significant reduction in mHtt aggregation. Rapamycin, a macrolide antibiotic, is approved for use in human patients. Employed in several clinical contexts, recent effectiveness has been demonstrated in cancer chemotherapy (Wendel *et al.*, 2004). More recent evidence demonstrated that rapamycin significantly improved neuronal survival, compared to wild-type flies. In addition, the rapamycin ester CCI-779 significantly improved motor performance and striatal neuropathology in the N171-82Q murine model of HD (Ravikumar *et al.*, 2004). In contrast, however, an inhibitor of autophagy, everolimus, had little effect in reducing mHtt levels, nor did it provide neuroprotection in the R6/2 HD mouse model (Fox *et al.*, 2010).

A screen of small molecules capable of modulating autophagy independent of mTOR has revealed a dozen small molecule enhancers of autophagy (SMERs), from which three positive hits (SMER 10, 18, and 28) were shown to reduce mHtt aggregation *in vitro*. was performed (Sarkar *et al.*, 2007). Further analysis demonstrated significant protection against mHtt toxicity in HD mice. While the precise therapeutic mechanism of SMERs remains unknown, an analysis of phosphorylation status in targets of mTOR showed no SMER-induced effects, suggesting the SMERs act downstream of mTOR to induce autophagy.

Interestingly, lithium has also been identified as a potential inducer of autophagy acting independently of mTOR, Lithium significantly has been shown to reduce clearance of mHtt and mHtt-induced cell death *in vitro* (Sarkar *et al.*, 2005). A similar mood stabilizing drug, carbamazepine, has also been shown to reduce mHtt-induced cell death and mHtt aggregation *in vitro*. The *in vitro* lithium-induced clearance of mHtt mediated by autophagy is independent of mTOR activity and dependent on inositol monophosphatase 1 (IMPase) activity, and the stimulatory effect is blocked with subsequent addition of inositol triphosphate. Interestingly, combined inhibition of mTOR and IMPase, by rapamycin and lithium, respectfully, resulted in additive clearance of mHtt *in vitro* (Sarkar *et al.*, 2005).

While strategies targeting enhanced clearance may promote improved neuronal survival, therapeutic attenuation of mutant protein aggregation may also prove therapeutically valuable in treating HD. In this regard, cystamine may hold significant promise. Cystamine is a disulfide-containing compound that possesses multiple modes of action, from antioxidant properties (Revesz and Modig, 1965), to inhibition of transglutaminase (Lorand *et al.*, 1978). Indeed, recent preclinical data from multiple laboratories has demonstrated the potential therapeutic benefit of cystamine in treating polyglutamine disorders, including HD (Dedeoglu *et al.*, 2002; Igarashi *et al.*, 1998; Karpuj *et al.*, 2002). More recently, the dimer of cystamine, cysteamine, a product of cystamine reduction, has completed Phase I human trials determining maximum dose tolerability and safety (Dubinsky and Gray, 2006). These data, in concert with previous clinical use of cysteamine for

treatment of cystinosis (McDowell *et al.*, 1998), demonstrate the unique potential of cystamine and its analogs in the treatment of HD.

Another approach in reducing huntingtin aggregation has been to use congo red. Congo red can inhibit huntingtin oligomerization and disrupt preformed oligomers, which prevents ATP depletion and caspase activation *in vitro* (Sanchez *et al.*, 2003). Intraperitoneal administration of congo red to R6/2 mice from 7 weeks of age improved survival by 16.4%. While a similar approach would be to use benzothiazoles, which prevent huntingtin aggregation *in vitro* (Heiser *et al.*, 2002), the *in vivo* efficacy of these compounds is yet to be established.

Huntingtin aggregates are a hallmark of both human HD and mouse models of HD and have been implicated as a plausible cause of neuronal death in HD. High throughput screening has identified a number of small molecule inhibitors of Htt aggregation, yielding a sulfobenzoic acid derivatives, C2-8, as a potential therapeutic lead (Chopra *et al.*, 2007). C2-8 has been shown to inhibit polyglutamine-mediated aggregation in cell culture and brain slices, and rescue photoreceptor degeneration in a *Drosophila* model of HD, while improving motor performance, reducing Htt aggregates, and ameliorating neuronal atrophy in R6/2 HD mice (Chopra *et al.*, 2007; Zhang *et al.*, 2005). ADMET profiling of 02-8 has been consistent with excellent drug-like properties. We have preliminary data showing that C2-8 significantly extends survival and reduces striatal neuron loss in R6/2 HD mice, while slowing motor dysfunction and Htt aggregate formation in the full-length 140 CAG HD mice throughout disease progression.

Lastly, it is of interest to note that therapeutic compounds not specifically directed at inhibiting mHtt have been shown to reduce mHtt aggregates (Dedeoglu *et al.*, 2003; Ferrante *et al.*, 2000, 2002; Gardian *et al.*, 2005; Klivenyi *et al.*, 2003; Stack *et al.*, 2006; Smith *et al.*, 2006; Wang *et al.*, 2003). These findings are largely the consequence of ameliorating disease progression. In contrast, there are agents that extend survival well beyond that reported in the above-mentioned studies, yet have little effect on Htt aggregates (Ferrante *et al.*, 2004). The antibiotic, mithramycin, is one such compound, extending survival almost 30% in the R6/2 HD mice.

### B. Transcriptional Dysregulation

Another profound aspect of disease pathology in HD is the alteration in gene transcription (Sugars and Rubinsztein, 2003). While the precise molecular basis for transcriptional dysregulation is unknown, there is significant evidence to suggest a direct interaction between the mHtt protein and transcription factors (Dunah *et al.*, 2002; Rubinsztein, 2003). Through sequestration of transcription factors into mHtt aggregates, it is thought mHtt brings about alterations in gene expression as observed in both human HD and murine models of HD (Augood *et al.*, 1997; Borovecki *et al.*,

2005; Cha *et al.*, 1999). Of great interest, transcriptional alterations associated with mHtt appear presymptomatically, suggesting such dysregulation is not an epiphenomenon. As such, there is now strong evidence that transcriptional dysfunction is related to histone hypoacetylation and hypermethylation in HD (Hake and Allis, 2006; Strahl and Allis, 2000). Experimental studies in murine models have demonstrated significant hypoacetylation of histone H4 (Ferrante *et al.*, 2003, 2004; Stack *et al.*, 2007b), while hypermethylation of histone H3 is observed in HD patients and HD mice (Ferrante *et al.*, 2004; Stack *et al.*, 2007b; Ryu *et al.*, 2006)

The transcriptional repression observed in HD likely results from alterations in chromatin packaging associated with epigenetic modifications of histone proteins. In general, therapeutic manipulation of transcription may offer significant benefit in treating HD, as well as other neurodegenerative disorders. In particular, pharmacological targeting of histone methylation and acetylation status may be a unique method by which to achieve transcriptional homeostasis, and by extension, neuroprotection in HD. Several preclinical trials with compounds directed toward altered histone profiles in HD have been performed. One strategy has been to target histone acetylation, by administering histone deacetylase inhibitors (HDACi) (Ferrante *et al.*, 2003; Steffan *et al.*, 2001).

The HDACi's sodium butyrate or suberoylanilide hydroxamic acid (SAHA) provide significant neuroprotection in a drosophila model of HD (Steffan *et al.*, 2001). These results were supported by *in vitro* analyses that demonstrated mHtt-induced inhibition of the histone acetyltransferase proteins CBP and p300 and improved the acetylation profile of histone H4 after sodium butyrate. Sodium butyrate and SAHA were also shown to provide neuroprotection in the R6/2 murine model of HD (Ferrante *et al.*, 2003; Hockly *et al.*, 2003). Sodium butyrate and SAHA improved motor performance, and while not reported for SAHA, sodium butyrate significantly improved survival (Ferrante *et al.*, 2003). Both compounds also markedly improved striatal morphology. Importantly, both sodium butyrate and SAHA improved acetylation of histone H4. Improvements in H4 acetylation mediated by sodium butyrate were concomitant with transcriptional improvements in R6/2 striatum, assessed by microarray gene profiling, resulting in improved mRNA expression (Ferrante *et al.*, 2003).

Confirming and expanding these data, the administration of the HDACi phenylbutyrate in the N171-82Q murine model of HD resulted in significant neuroprotection as well (Gardian *et al.*, 2005). Of interest, in addition to phenylbutyrate-mediated improvements in H4 acetylation, there was also a significant reduction in methylation of H3 within the striatum. Together, these data clearly link HDACi treatment with improved transcription. With the prospect of HDACi compounds offering therapeutic benefit in the treatment of HD, a dose-finding study using sodium phenylbutyrate recently demonstrated that doses ranging from 12 to 15 g/d were safe and well tolerated (Hogarth *et al.*, 2007). Our own preliminary data using 15 g/d in a safety and tolerability trial in HD patients confirms the

above study and, in addition, showed that sodium phenylbutyrate was therapeutically salient in significantly improving hypoacetylation levels in blood buffy-coat specimens in the trial subjects.

In addition to compounds that directly interact with histone deacetylases, another class of potential HD therapeutics exist that interact directly with DNA and may potentially influence transcriptional activity in HD. Two such compounds are mithramycin and chromomycin, anthracycline antibiotics that act through modulating gene transcription. By binding to guanine-cytosine-rich regions within gene promoters, anthracyclines displace transcriptional elements that activate and repress transcription (Chakrabarti *et al.*, 2000). Importantly, anthracyclines have been reported to interact directly with histones H3 and H4 (Rabbani *et al.*, 2004). This has lead to several preclinical studies investigating the potential utility of mithramycin or chromomycin in murine models of HD (Ferrante *et al.*, 2004; Ryu *et al.*, 2006; Stack *et al.*, 2007b). Mithramycin administration in R6/2 HD mice resulted in the largest significant extension in survival (29.1%) compared with any other preclinical therapeutic trial in HD to date (Ferrante *et al.*, 2004). The mithramycin-mediated improvement in survival was concomitant with significant improvements in motor performance and striatal morphology. Notably, mithramycin induced a significant decrease in methylated H3. In a follow-up study, mithramycin-mediated improvements were found in the methylation and acetylation profile of H3 and H4 within the striatum of N171-82Q mice (Stack *et al.*, 2007b). Treatment with chromomycin or mithramycin in R6/2 and N171-82Q HD mice significantly increased acetylation of H4, and significantly reduced trimethylation of H3 at lysine 9. Additional analyses of H3 revealed an anthracycline-mediated shift toward greater acetylation and reduced methylation compared with untreated controls. This latter finding may be of particular importance given that methylation of H3 at lysine 9 is thought to be a dominant marker of transcriptional repression, and the balance between methylation and acetylation of H3 at lysine 9 is believed to play an important role in the transcriptional disruption observed in HD (Ryu *et al.*, 2006).

While it may seem incongruous that cytotoxic antitumor compounds play a positive role in neurodegenerative disorders, parallels between cancer and neurodegenerative disorders have been suggested. However, if cellular stresses and transcriptional signals elicit different responses in dividing cells versus cells that are terminally differentiated (leading to oncogenesis in the former and neurodegeneration in the latter), then different pathogenic mechanisms may underlie each. Importantly, previous clinical use of these compounds has been associated with negative side effects, including fever, nausea or vomiting, fatigue, and depression. Both agents cross the blood–brain barrier and mithramycin has been used chronically in a number of human conditions. The preclinical mithramycin and chromomycin data provide a rationale for clinical trials of these approved anthracyclines to test for efficacy in the treatment of HD.

### C. Oxidative Stress and Mitochondrial Dysfunction

In disease-free neurons, the generation of reactive oxygen species is a normal byproduct of cellular respiration that is mediated by mitochondria. Accumulation of reactive oxygen species in neurons and subsequent oxidative stress is blocked by free radical scavengers, such as glutathione and superoxide dismutase, preventing subsequent damage (Beal, 1998, Beal, 1999; Calabrese *et al.*, 2006; Mancuso *et al.*, 2007). In HD, the generation of reactive oxygen species and the resulting oxidative stress is thought to play a central role in the neurodegeneration observed (Beal, 1992; Browne *et al.*, 1997; Ferrante *et al.*, 1994; Grunewald and Beal, 1999; Ross and Poirier, 2004). There are multiple lines of evidence implicating oxidative stress in the etiology of neuronal death in HD (Grunewald and Beal, 1999). Studies from postmortem human HD brain show increased levels of oxidative damage. These include increased cytoplasmic lipofuscin, DNA strand breaks, and the accumulation of oxidative markers in DNA bases, along with other cellular macromolecules associated with protein nitration and lipid oxidative damage.

Lipofuscin is an aging wear-and-tear pigment and is the product of unsaturated fatty acid peroxidation that is increased at a greater rate under oxidative stress (Sohal and Brunk, 1989; Terman and Brunk, 1998). There is an abnormal accumulation of lipofuscin in HD patients within both cortical and striatal neurons in HD patients (Braak and Braak, 1992; Browne *et al.*, 1999; Tellez-Nagel *et al.*, 1974), with little or no presence of lipofuscin in spared NADPH-diaphorase neurons in the caudate nucleus (Browne *et al.*, 1999). Mutant proteins are processed through lysosomes and, as such, the lipofuscin accumulation has been suggested to impair lysosomal function, resulting in selective neuronal damage.

DNA strand breaks are related to free radical damage (Driggers *et al.*, 1997) and have been reported to be increased in HD patients, correlating with CAG repeat length (Browne *et al.*, 1999; Butterworth *et al.*, 1998; Dragunow *et al.*, 1995; Portera-Cailliau *et al.*, 1995). Using *in situ* end labeling, which identifies DNA fragmentation in apoptotic ornecrotic nuclei, significant increases in DNA fragmentation in both striatal and cortical neurons in HD patients, relative to levels of DNA fragmentation in age-matched control brains (Browne *et al.*, 1999). In addition, it may be that mitochondrial DNA is more susceptible than nuclear DNA to fragmentation since there is less *in situ* end labeling detected within cell nuclei.

The oxidation of either nuclear or mitochondrial DNA results in the formation of the metabolite 8-hydroxy-2'-deoxyguanosine ($OH^8dG$) and is a direct result of free radical activity (Browne *et al.*, 1997; Dragunow *et al.*, 1995; Polidori *et al.*, 1999). Significant increases in $OH^8dG$ levels from nuclear DNA occur in the caudate nucleus in postmortem tissue from HD patients (Browne *et al.*, 1997), as well as in mitochondrial DNA from parietal cortex (Polidori *et al.*, 1999). In addition, $OH^8dG$ levels are markedly elevated in serum from HD patients (Hersch *et al.*, 2006) and, as such, provide a peripheral biomarker as an indicator

of therapeutic response (Hersch *et al.*, 2006). These findings are consistent with elevations of $OH^8dG$ levels that occur in other neurodegenerative diseases in which oxidative damage has been implicated as a pathogenic mechanism (Ferrante *et al.*, 1997; Mecocci *et al.*, 1993). Others, however, have not observed changes in nuclear DNA in HD patients (Alam *et al.*, 2000).

In parallel, additional markers of oxidative damage, including heme oxygenase, 3-nitrotyrosine and malondialdehyde are all elevated in both HD striatum and cortex (Browne *et al.*, 1999), (Ferrante *et al.*, 1996). The extent and intensity of these markers mirror the dorsal-ventral pattern of progressive neuronal loss in the neostriatum, with increased immunoreactive expression in the dorsal striatum, in comparison to the less severely involved ventral striatum. Consonant with the immunohistochemical data, analysis of colorimetric assays in HD patients show significant increases in malondialdehyde and 4-hydroxynononeal brain levels, almost eightfold greater than in control subjects (Stoy *et al.*, 2005).

Many of the oxidative alterations observed in human HD are recapitulated in genetic murine models of HD, making them ideal vehicles in which to study pathogenesis and therapeutic potential. In the R6/2 transgenic model of HD, which expresses the N-terminal fragment of mHtt containing the CAG repeat (Mangiarini *et al.*, 1996) and shows striatal neuronal loss (Stack *et al.*, 2005), there is a significant increase in brain and urinary $OH^8dG$ levels (Stack *et al.*, 2005), (Bogdanov *et al.*, 2001), arguing that oxidative stress may be the consequence of mHtt expression. In addition, it has been shown that there are increases in lipid peroxidation that worsen with disease progression in these mice (Browne and Beal, 2006). Malondialdehyde, 4-hydroxynonenal, and the isoprostane, 8-iso-prostaglandin, are all elevated in R6/2 mice at disease onset and increase with disease progression (Browne and Beal, 2006), along with increased immunostaining for inducible nitric oxide synthase and nitrotyrosine (Tabrizi *et al.*, 2000). In the less fulminant R6/1 transgenic HD mice, there is a progressive increase in striatal lipid peroxidation that parallels the progression of the neuropathological phenotype (Perez-Severiano *et al.*, 2000). Additional evidence of alterations in oxidative stress come from studies in 140CAG full-length knock-in mice (Menalled *et al.*, 2003). In contrast to segment models of HD, mice containing the full-length huntingtin gene provide the best possible molecular genetic comparison to human HD. We have evidence that $OH^8dG$ urine and brain levels are significantly elevated in the 140CAG mice. As in human HD, the mouse data provide a direct link between mHtt expression, metabolic dysfunction, and the generation of reactive oxygen species and oxidative stress.

The primary source of reactive oxygen species in neurons is mitochondria and, as such, mitochondrial dysfunction in HD is intimately associated with oxidative stress. Mitochondria are a vital component of the cell, generating energy for all molecular processes and regulating cellular function (Benard *et al.*, 2007). Impairment of mitochondria leads to a cascade of events that include increased

production of reactive oxygen species, reduced ATP production, altered calcium homeostasis, and cytochrome c release and apoptosis that subsequently lead to neuronal death (Lee and Wei, 2000; Wang *et al.*, 2009). Neurons are especially vulnerable to mitochondrial abnormalities as they have a high metabolic demand and extraordinarily high energy requirements (Kann and Kovacs, 2007). There is strong evidence from morphologic and biochemical studies that mitochondrial dysfunction plays a prominent role in the pathogenesis of HD (Kim *et al.*, 2010a; Lin and Beal, 2006). Biochemical analysis of the HD brain reveals reduced glucose metabolism, reduced striatal glucose utilization proceeding tissue loss (Jenkins *et al.*, 2000; Kuhl *et al.*, 1982), decreased mitochondrial complex activity, and increased lactate concentration (Koroshetz *et al.*, 1997). In addition, mitochondria from HD patients have been shown to have lower membrane potentials and to require lower calcium loads for depolarization (Panov *et al.*, 2002). In conjunction with this, biomarkers of oxidative stress have been found to be elevated in HD human and mouse brain and serum including markers of DNA oxidative modification, strand breaks (Bogdanov *et al.*, 2001, Hersch *et al.*, 2006, Mecocci *et al.*, 1993) and deletions in mitochondrial DNA (Polidori *et al.*, 1999).

Mutant huntingtin protein is thought to be directly involved in mitochondrial dysfunction by means of transcriptional dysregulation of nuclear-encoded mitochondrial genes. Mutant Htt causes transcriptional dysregulation of TATA-binding proteins and Sp1 that results in an alteration of nuclear-encoded mitochondrial genes. Support for this comes from studies on peroxisome proliferator-activated receptor- γco-activator (PGC-1α), a transcriptional co-activator that regulates mitochondrial gene expression, the production of antioxidant enzymes, mitchochondrial uncoupling proteins, and is important in the regulation of ATP (Lin *et al.*, 2004, 2005; Rohas *et al.*, 2007). PGC-1α is repressed by mHtt leading to mitochondrial dysfunction (Cui *et al.*, 2006). There is recent evidence showing significant reductions of PGC-1α and the nuclear-encoded transcription factor TFAM in muscle biopsies and myoblast cultures from HD patients (Chaturvedi *et al.*, 2009). Additionally, we have recently shown a grade-dependent reduction of both PGC-1α and TFAM in the brain lysates of HD patients. Further evidence supporting PGC-1α's role in mitochondrial impairment comes from studies done in R6/2 mice where reduced levels of PGC-1α result in striatal neurodegeneration and motor abnormalities in HD mice, along with increased sensitivity to oxidative stressors. The delivery of lentiviral-mediated PGC-1α expression into the striatum of R6/2 mice significantly improved the pathological phenotype.

Finally, there is evidence of morphological and biochemical alterations in mitochondrial fission and fusion. Mitochondria are distributed throughout the cells forming individual units or interconnected networks. The distribution of mitochondria is dynamic and mitochondria are trafficked throughout the cell by modulating their shape and size through mechanisms of fission and fusion. In a normal cell, there is an equilibrium between fission and fusion; however, any

alteration in this dynamic leads to mitochondria dysfunction and mitophagy (Chen and Chan, 2009) and therefore may trigger cell death. There is experimental evidence that this dynamic may be altered in HD. Drp1 and Fis1 are the principal arbiters of mitochondrial fission and fusion proteins. Altered levels of these proteins have been reported in HD caudate nucleus lysates, consistent with altered mitochondrial dynamics, mitochondrial distribution, and trafficking in HD and confirmed by histopathological analysis (Kim *et al.*, 2010a). These findings suggest that altered fission and fusion dynamics may be an important mechanism leading to mitochondrial dysfunction and subsequent neuronal loss in HD (Knott and Bossy-Wetzel, 2008).

Given the role of oxidative stress associated with mitochondrial dysfunction, several preclinical antioxidant strategies have been employed with promising success. First, among these is the guanidine compound creatine which, while produced endogenously, is also obtained from the diet (Ryu *et al.*, 2005). In addition to its antioxidant capacity, creatine also buffers intracellular energy reserves, stabilizes intracellular calcium, and inhibits activation of the mitochondrial transition pore (O'Gorman *et al.*, 1997). In neurons, creatine can exist as free substrate, or phosphocreatine (PCr). According to the PCr shuttle hypothesis, sites of energy production are connected with sites of energy consumption when creatine kinase mediates the transfer of a phosphoryl group from PCr to ADP, creating ATP (Bessman and Geiger, 1981). In HD, there is a significant shift in the ratio of PCr to phosphate (Koroshetz *et al.*, 1997). Thus, creatine administration may be able to restore normal metabolic activity. To this end, several preclinical studies have provided ample evidence of the neuroprotective benefit of creatine in chemical and animal models of neurodegenerative disease, including HD (Andreassen *et al.*, 2001; Dedeoglu *et al.*, 2003; Ferrante *et al.*, 2000; Klivenyi *et al.*, 1999; Matthews *et al.*, 1998a, 1999; Zhu *et al.*, 2004).

In the R6/2 mouse, creatine significantly improves survival and motor performance, ameliorates brain and striatal atrophy, and reduces striatal mHtt aggregation in a dose-dependent manner. Oral creatine administration also increases brain levels of creatine. This effect has been confirmed in another animal model of HD (Andreassen *et al.*, 2001), suggesting the significant promise of creatine administration in the treatment of HD. Several clinical trials show safe and tolerable doses of creatine in HD patients ranging from 5 to 10 g/d (Bender *et al.*, 2005; Hersch *et al.*, 2006; Tabrizi *et al.*, 2005; Verbessem *et al.*, 2003). Creatine treatment in human HD resulted in a significant reduction in brain glutamate (Bender *et al.*, 2005) and oxidative stress, as measured by 8-hydroxy-2'-deoxyguanosine (8OH2'dG) (Hersch *et al.*, 2006). The 8OH2'dG findings are the first instance of parallel efficacy using a common peripheral biomarker in the administration of a therapeutic agent in HD mice and HD patients. No studies, however, have been sufficiently powered to detect a significant slowing of progression or improvement

in clinical measures. Although in a 1-year open-label pilot study, creatine (10 g/d) administered for 12 months resulted in unchanged Unified Huntington Disease Rating Scale (UHDRS) scores, suggesting that creatine may be effective in stabilizing disease progression (Tabrizi *et al.*, 2003). Although the optimal dose of creatine is not yet certain, it is possible that the dose of creatine supplementation in the above studies may have been underestimated.

The effects of a high-dose creatine administration in multiple murine models of HD has recently been studied (Foran *et al.*, 2006). High-dose creatine administration was well tolerated by both R6/2 and 140CAG mice, and at dosages >200% of previously successful preclinical dosing strategies (Ferrante *et al.*, 2000), we demonstrated significant improvement in survival and motor performance. Further analysis revealed significant improvements in striatal neuropathology, with concomitant reductions in both mHtt aggregation and 8OH2'dG levels. In addition, there was a significant creatine-mediated improvement in striatal ATP levels. While drug trials in mice confirm therapeutic direction, the challenge is in determining what dose might be of value in patients since the pharmacokinetics of mice and man is dissimilar. As such, a much higher dose may be feasible for humans. In this regard, a dose escalation study up to 40 g/d to determine whether there is a maximally tolerated dose in HD, as well as whether there are doses at which serum and brain levels of creatine are maximized, has been initiated. Preliminary results suggest that creatine (20–30 g/d) is effective in slowing disease progression.

Another antioxidant compound that has demonstrated preclinical efficacy in multiple murine models of HD is coenzyme $Q_{10}$ (Beal *et al.*, 1994; Ferrante *et al.*, 2002; Schilling *et al.*, 2001; Stack *et al.*, 2006). Coenzyme $Q_{10}$ ($CoQ_{10}$), also known as ubiquinone, is a lipid-soluble benzoquinone that possesses significant antioxidant properties when reduced to ubiquinol, or through a $CoQ_{10}$-induced increase in alpha-tocopherol (Beal and Ferrante, 2004). It is located in the inner mitochondrial membrane and is essential for Complex I and II electron transfer activities during oxidative phosphorylation (Chan *et al.*, 2004), playing a vital role in ATP production. Importantly, $CoQ_{10}$ administration has been demonstrated to significantly increase brain mitochondrial $CoQ_{10}$ concentrations (Matthews *et al.*, 1998b). Initial preclinical therapeutic trials using $CoQ_{10}$ in a striatal lesion model of HD demonstrated significant neuroprotection (Beal *et al.*, 1994). Malonate-induced lesions within the striatum were significantly reduced by $CoQ_{10}$. Expanding these results, others and we conducted preclinical therapeutic trials using $CoQ_{10}$ in murine models of HD (Ferrante *et al.*, 2002; Schilling *et al.*, 2001). $CoQ_{10}$ treatment significantly extends survival and delays the typical decline in weight loss and motor performance as assessed on the rotarod. In addition, $CoQ_{10}$ administration significantly attenuates brain weight loss, gross brain atrophy and ventricular enlargement, and striatal neuron atrophy. These data have given way to several human safety and tolerability trials using $CoQ_{10}$ (Feigin *et al.*, 1996; Huntington Study Group, 2001; Koroshetz *et al.*, 1997).

In all instances, $CoQ_{10}$ has been found to be both safe and tolerable in HD patients. $CoQ_{10}$ treatment has resulted in a significant decrease in cortical lactate (Koroshetz *et al.*, 1997), as well as a nonsignificant trend toward slowing in total functional capacity decline over 30 months (Huntington Study Group, 2001). In addition, there were significant beneficial effects on cognitive function, including Stroop color naming and word reading tasks (Huntington Study Group, 2001). Since the single target dose, however, did not provide significance in the specified primary outcome of the trial, it remains unclear whether a higher $CoQ_{10}$ dose would provide greater efficacy in HD patients. A number of studies in other neurodegenerative diseases suggest that a higher $CoQ_{10}$ dose is possible. A double-blind, randomized, controlled trial in Parkinson's disease (PD) patients, using $CoQ_{10}$ at 1200 mg/d, slowed the rate of deterioration in the Unified PD Rating Scale score (Shults *et al.*, 2002). Follow-up studies in both PD and amyotrophic lateral sclerosis patients have demonstrated safe and tolerable doses up to 3000 mg/d (Ferrante *et al.*, 2005; Shults *et al.*, 2004).

Addressing this, a high-dose trial using $CoQ_{10}$ in R6/2 mice has been performed (Smith *et al.*, 2006), with dosages 10 times those previously reported (Beal *et al.*, 1994; Ferrante *et al.*, 2002). High-dose $CoQ_{10}$ treatment in R6/2 resulted in significant survival extension with significant improvements in motor performance. As with previous preclinical trials using $CoQ_{10}$, high-dose $CoQ_{10}$ treatment in R6/2 resulted in improved neuropathology, with marked reductions in striatal mHtt aggregation. Coupled with significant improvement in brain ATP levels and a reduction in brain 8OH2'dG, these results demonstrate the pluripotent efficacy of high-dose $CoQ_{10}$ in the treatment of HD. A multicenter Phase II-III clinical trial using high-dose $CoQ_{10}$ has been initiated.

Therapies targeting alternative aspects of mitochondrial function may also be effective. In this regard, the *n*-3 fatty acid eicosapentaenoic acid (EPA) possesses hypotriglyceridemic activity, shown to occur through EPA interactions with mitochondria (Froyland *et al.*, 1997), acting as a mitochondrial proliferator. EPA-induced hippocampal neuroprotection has been observed in rats treated with whole body γ-irradiation (Lonergan *et al.*, 2002), by significantly reducing reactive oxygen species, cytochrome *c* translocation, and caspase-3 activation. Importantly, mitochondrial dysfunction in HD mediated by mHtt has been shown to promote altered calcium permeability and associated cytochrome *c* release (Choo *et al.*, 2004). The ability of EPA to interact with and promote mitochondrial fitness has stimulated interest in EPA as a potential therapy for the treatment of HD.

Using a purified derivative of EPA known as Miraxion or ethyl-EPA, an animal trial in the R6/1 murine model of HD showed significant improvements in multiple motor and behavioral abnormalities (Clifford *et al.*, 2002). A subsequent 6-month clinical trial using ethyl-EPA in advanced HD patients demonstrated significant improvement in several orofacial aspects of the UHDRS (Puri *et al.*, 2002). The improvements in the UHDRS were concomitant with improved

neuropathology, assessed through MRI. More recently, however, ethyl-EPA treatment in HD patients was reported to have no effect on the UHDRS (Puri *et al.*, 2005). While secondary analysis revealed ethyl-EPA-induced improvements in motor function, further studies will be required to determine the therapeutic potential of ethyl-EPA.

The antihistamine Dimebon (2,3,4,5-tetrahydro-2,8-dimethyl-5-(2-(6-methyl-3-pyridyl)ethyl)-1H-pyrido(4,3-b) indole), is an orally active small molecule that has multiple mechanisms of action. It may exert a neuroprotective effect by interacting with the mitochondrial permeability transition pore and preventing the calcium-induced opening of the pore (Bachurin *et al.*, 2003). Studies of Dimebon in animal models of Alzheimer's disease have showed improved cognitive ability, while inhibiting beta-amyloid (Bachurin *et al.*, 2001). Preliminary results in Alzheimer's disease patients have also been promising. That Dimebon may also regulate calcium homeostasis and reduce the excitotoxicity (below), there may be potential benefit in administering Dimebon to HD patients. As such, a Phase II clinical trial using Dimebon (latrepirdine) in HD patients showed no significant treatment effects on the UHDRS or the ADAS-cog (Kieburtz *et al.*, 2010).

### D. Excitotoxicity

In HD, excessive glutamatergic input to the striatum is hypothesized to contribute to the striatal neurodegeneration observed. Evidence supporting the excitotoxic hypothesis stems from observations of similarities between kainic, glutamic, and quinolinic acid lesions and the striatal pathology observed in rodent and primate models of HD (Beal *et al.*, 1991b; Coyle and Schwarcz, 1976; Ferrante *et al.*, 1993; McGeer and McGeer, 1976). Increases in striatal glutamate in the brains of HD patients (Taylor-Robinson *et al.*, 1996) as well as alterations in presynaptic glutamate receptors in the R6/2 murine model of HD (Cha *et al.*, 1999) lend additional support to the role of aberrant glutamate excitotoxicity in HD pathogenesis. Given the fact that increased levels of excitatory amino acids are not elevated in HD, the concept of slow excitotoxicity in HD was suggested by Albin and Greenamyer, and Beal as an alternative excitotoxic hypothesis in which normal circulating levels of glutamate could result in neuronal dysfunction and death (Albin and Greenamyre, 1992; Beal, 1992). It is of interest to note, as discussed above, both surgical and chemical lesions of the corticostriatal and nigrostriatal pathways, improve the pathological phenotype in R6/2 transgenic mice and extend survival in this HD mouse model (Stack *et al.*, 2007a).

Given extensive evidence in support of an excitotoxic hypothesis for HD, compounds that counter excessive glutamate release may therefore be candidates for therapeutic intervention in HD. One such FDA-approved compound is riluzole (2-amino-6-trifluoromethoxy benzothiazole), a potent anti-glutamatergic agent,

attenuates glutamate release through its ability to inhibit voltage-dependent sodium channels (Urbani and Belluzzi, 2000). In HD, the potential benefit of riluzole was first suggested by preclinical studies in rats and nonhuman primates, using the 3-nitroproprionic (3-NP) chemical model of HD (Guyot *et al.*, 1997b; Palfi *et al.*, 1997). In the 3-NP model of HD, riluzole offers significant improvements in motor performance, with significant neuroprotection observed. Expanding these findings, riluzole was found to significantly increase survival in R6/2 mice concomitant with significant improvements in motor behavior (Schiefer *et al.*, 2002). There was also a marked riluzole-mediated reduction in ubiquitin-positive mHtt aggregates within the striatum. Riluzole was also found to protect medium spiny neurons against glutamate-induced apoptosis *in vitro* (Wu *et al.*, 2006). The aberration in corticostriatal function resulting in excessive glutamate release is widely thought to contribute to the selective striatal pathology observed in HD (Beal, 1992; Olney, 2011). Interestingly, riluzole administration significantly reduces aberrant excitatory postsynaptic currents in R6/2 mice, lending further support for riluzole therapy in HD (Cepeda *et al.*, 2003). As such, several clinical trials in human HD have been conducted. In a 6-week safety and tolerability trial with riluzole that assessed motor performance and brain lactate levels (Rosas *et al.*, 1999), riluzole was found safe and well tolerated, with a nonsignificant trend toward lower basal ganglia lactate levels. Analysis of motor function demonstrated a significant decrease in chorea, as measured via the UHDRS; however, subsequent studies have shown this effect to be transient (Rosas *et al.*, 1999; Seppi *et al.*, 2001). A Phase III study was recently conducted in Europe based on data suggesting that riluzole upregulated levels of neuroprotective factors. The study, conducted over 3 years recruited 400 early symptomatic patients (Mizuta *et al.*, 2001) and found riluzole to have no beneficial effect on symptoms or neuroprotection, nor did it halt or slow progression of disease (Landwehrmeyer *et al.*, 2007). A more recent study of riluzole in HD, however, reported reduced gray matter volume loss and brain glucose hypometabolism, along with increasing neurotrophin production, providing evidence in support of riluzole (Squitieri *et al.*, 2009). Interestingly, the efficacy of other glutamatergic NMDA antagonists, amantadine (Verhagen Metman *et al.*, 2002), and memantine (Ondo *et al.*, 2007) has had mixed results (Heckmann *et al.*, 2004; Lipton, 2004; Lucetti *et al.*, 2003; O'Suilleabhain and Dewey, 2003). Each of these drug agents is associated with significant side effects. Memantine, a glutamatergic NMDA antagonist, may hold more promise. Memantine blocks NMDAR and reduces striatal cell death in chemical models of HD (Lee *et al.*, 2006). An open-label study suggested that memantine may slow down disease progression and anecdotal reports have suggested that it may lead to improvements in cognition (Cankurtaran *et al.*, 2006). Based on these results and the beneficial effects of memantine in other dementing conditions, a Phase IV clinical study has been initiated to determine the effect of memantine on memory, cognition, and behavior in HD patients.

## E. Apoptosis

Alterations in apoptotic signaling cascades have been shown to play a role in the pathogenesis of HD. In apoptotic-induced cell death, signaling cascades activate proteases that destroy proteins essential for survival and concurrently activate genes involved in cell suicide. The primary constituents of the apoptotic cascade are caspases, a class of cystenine proteases. There are four initiator caspases and three effector caspases including caspase-3, -6, and -7 (Friedlander, 2003). One important event in the apoptotic cascade is the release of cytochrome *c* by mitochondria into the cytoplasm which activates caspase-9, leading to the subsequent activation of down-stream executioner caspases and eventual cell death (Chen *et al.*, 2000).

*In vitro* and *in vivo* studies have shown that wild-type huntingtin has an antiapoptotic role. In one experiment, brain-derived cells overexpressing wild-type huntingtin were found to be less sensitive to toxic stimuli (Rigamonti *et al.*, 2001, 2007a). In another study, primary striatal neurons from YAC18 mice overexpressing full-length wild-type huntingtin were shown to be protected from apoptosis compared to littermate wild-type controls and YAC72 mice expressing mutant huntingtin (Leavitt *et al.*, 2006). Finally, cells with lower levels of wild-type huntingtin were shown to be more sensitive to apoptotic cell death and had increased levels of caspase-3 activity (Zhang *et al.*, 2006). Although the exact mechanism by which wild-type huntingtin exerts its antiapoptotic effects is unknown, experimental evidence has suggested that wild-type huntingtin blocks the formation of a functional apoptosome complex thereby preventing subsequent activation of caspase-3 and caspase-9 (Rigamonti *et al.*, 2001, 2007b). Additionally, evidence suggests that wild-type huntingtin physically interacts with active caspase-3, inhibiting its proteolytic activity (Zhang *et al.*, 2006). Intracellular calcium dysfunction has been found to contribute to apoptosis induced cell death in HD. One mechanism of this dysfunction is mutant huntingtin's interaction with the NMDA receptor (NMDAR) resulting in its overactivation. Increased activation of NMDAR leads to increased calcium levels in the cytosol resulting in mitochondrial calcium overload causing mitochondrial swelling and the release of cytochrome *c* and other proapoptotic factors into the cytoplasm. In addition to enhancing NMDAR function, mutant huntingtin binds to the type 1 inositol 1,4,5-triphosphate receptors ($InsP_3R1$) making it more sensitive to $IP_3$ and causing more calcium to be released. Additionally, mutant huntingtin acts on the mitochondrial transition pore to decrease the calcium threshold needed to trigger the pore opening.

As increased caspase activity has been shown to contribute to the pathogenesis of HD, compounds that inhibit caspases have been tested as therapeutic candidates. One such compound is minocycline, a second-generation tetracycline antiapoptotic compound that inhibits caspase-1 and caspase -3 activity and expression

levels, the release of apoptogenic factors from mitochondria, and caspase-independent neuronal cell death pathways. Additionally, it may inhibit iNOS activity and reactive microgliosis, both of which have been found to be present in HD patients and HD mice and have been implicated in disease pathogenesis (Friedlander, 2003). Previous research has found minocycline to be neuroprotective in multiple experimental models of neurodegeneration including brain trauma, cerebral ischemia, amyotropic lateral sclerosis, and Parkinson's disease (Chen *et al.*, 2000; Du *et al.*, 2001; Sanchez *et al.*, 2003; Tikka *et al.*, 2001; Wu *et al.*, 2002; Yrjanheikki *et al.*, 1998; Zhu *et al.*, 2002). Importantly, it is able to cross the blood–brain barrier and has been found to be safe for chronic administration (Domercq and Matute, 2004).

Among anti-apoptotic drug candidates, the tetracycline antibiotic minocycline has emerged as a potentially beneficial therapeutic intervention for treatment in HD. Minocycline possesses potent anti-apoptotic capacity through inhibitory effects on caspases –1 and –3. In addition, minocycline also attenuates disruptions in mitochondrial function, including the release of cytochrome *c* (Chen *et al.*, 2000; Zhu *et al.*, 2002). Importantly, minocycline also readily crosses the blood–brain barrier. Clinically, chronic administration of minocycline has yielded a good safety record (Domercq and Matute, 2004). From a therapeutic standpoint, minocycline has shown significant improvement in multiple models of neurodegeneration, including brain trauma, spinal cord injury, PD, and HD (Chen *et al.*, 2000; Du *et al.*, 2001; Stack *et al.*, 2006; Teng *et al.*, 2004; Wang *et al.*, 2003; Yrjanheikki *et al.*, 1998).

In preclinical trials using minocycline in murine models of HD, several studies have demonstrated a significant neuroprotection. Minocycline significantly inhibited caspase-1 and caspase-3 activation in R6/2 mice (Chen *et al.*, 2000). Minocycline also significantly reduced mHtt cleavage. In addition to their role in apoptotic signaling cascades, caspases also play a role in cleaving mHtt, yielding the toxic fragment (Sawa *et al.*, 2005). Inhibition of caspase activity was associated with improved survival and motor behavior in R6/2 mice. Extending these findings, minocycline has been shown to significantly inhibit both initiator and effector caspases, including caspase-1, -3, -8, and -9, as well as the pro-apoptotic Bid cleavage (Wang *et al.*, 2003). In addition, minocycline also inhibited both the release of cytochrome *c* and Smac/Diablo from mitochondria in R6/2 mice, indicating that mitochondria are a direct target of minocycline-mediated neuroprotection (Teng *et al.*, 2004; Wang *et al.*, 2003; Zhu *et al.*, 2002).

These preclinical minocycline studies have given way to pilot clinical trials assessing safety and tolerability in human HD. At doses of 100 and 200 mg/d, minocycline was well tolerated by patients (Huntington Study Group, 2004). In terms of cognitive outcomes, there were no clinically relevant differences in cognition assessed by UHDRS. Similar results in pilot trials using minocycline at 100 mg/d over 6 months have been reported (Bonelli *et al.*, 2003), (Thomas *et al.*,

2004). There is excellent safety and tolerability data for minocycline treatment in HD patients. While a clinical trial in amyotrophic lateral sclerosis using 400 mg/d showed no efficacy (Traynor *et al.*, 2006), it has been suggested that the target dose was too great, resulting in the negative findings. Nevertheless, a recent futility study using minocylcine (200 mg/d) in HD patients suggested that further study of minocycline in HD was not warranted (Huntington Study Group DOMINO Investigators, 2010). While the initial analysis indicated that futility was not declared, a secondary analysis showed futility. These findings, however, used a 25% threshold compared to a fixed value from a historical database.

In addition to minocycline-mediated caspase inhibition, a recent report demonstrated a novel reversible inhibitor of caspase-3 that was shown to provide significant neuroprotection in a chemical rat model of HD (Toulmond *et al.*, 2004). In a preclinical proof-of-principle trial, M826, a pyrazinone mono-amide, demonstrated significant protection against malonate lesions, with a pharmacokinetic profile indicating the ability of M826 to inhibit capsase-3 6 h postadministration. Striatal lesion volumes were significantly reduced following M826 administration, and the number of neurons expressing active caspase-3 was also significantly reduced. While these results demonstrate significant neuroprotective potential, the route of administration (intracerebral injection) will require additional study to improve and assess both M826 solubility and brain penetration *in vivo* (Han *et al.*, 2005; Toulmond *et al.*, 2004).

There are several other therapeutic approaches that may prove beneficial in treating HD, and thus deserve mention here.

### F. RNA Interference (RNAi)

RNAi is one such therapy that takes advantage of a functionally conserved pathway present in all eukaryotes (Sah, 2006). The molecular machinery mediating RNAi activity includes both micro RNA (miRNA) and short interfering RNA (siRNA). Through associations between various proteins, including Argonaute-2 and individual RNAi molecules, a functional complex is formed that can then target homologous mRNA. Once bound to the homologous mRNA species, Argonaute-2 cleaves, and thus inactivates, the homologous mRNA (Liu *et al.*, 2004). Both miRNA and siRNA can prevent translation of homologous mRNA when each possesses a limited number of mismatches (Zeng *et al.*, 2003). Through this mechanism, RNAi could be manipulated to reduce expression of protein products known to cause disease. In the case of HD, the ability to effectively target and down regulate mHtt expression may hold significant promise. HD is an especially good candidate for gene silencing therapy because the mutant gene causes production of a presumably single toxic molecule that causes the disease phenotype.

In this regard, several preclinical studies have shown the potential promise of RNAi therapy in HD. Using an adeno-associated viral vector (AAV) expressing a short hairpin RNA precursor targeting the Htt gene, mHtt expression is reduced in the striatum of N171-82Q mice (Harper *et al.*, 2005). RNAi targeting mHtt also improved motor behavior, with improved gait and rotarod performance. Similar results were also obtained in the R6/1 murine model of HD (Rodriguez-Lebron *et al.*, 2005). Although the success seen in these experiments is very promising, there are remaining concerns before tRNAi therapy can be translated to humans. First, is the inability to silence mutant huntingtin without also affecting wild-type huntingtin. Attempts have been made to selectively silence mutant huntingtin by targeting the expanded polyglutamine sequences; however, this method has proven ineffective. Studies in knock-in models of mice, however, have shown a reduction in the expression levels of mutant and wild-type huntingtin without deleterious effects (McBride, 2008). Future work may target the reduction level in both mutant and wild-type Htt that provides the greatest efficacy on symptoms, while maintaining the neuroprotective effects of wild-type Htt. A second concern using RNAi therapy is the method of delivery. While intracerebral infusion may be applicable in experimental animals, safe and effective delivery of RNAi molecules to humans has yet to be firmly established.

Another method of gene silencing is antisense oligonucleotide gene inactivation. Similar to RNAi, this method inactivates mRNA, therefore preventing the translation of proteins. The mechanism of inactivation, however, is different. Synthetic single strands of 15–25 oligonucleotides are engineered to have a sequence complimentary to the mHtt mRNA and injected into the cell. Once in the cell, the oligonucleotides bind the mRNA and inhibit production of the protein by either physically blocking translation or by recruiting the enzyme RNase H to degrade the mRNA. One advantage of antisense gene therapy over RNAi is that oligonucleotides are smaller than RNAis, being single stranded instead of double stranded, and are therefore easier to get into the cell. However, the method of delivery to the CNS remains difficult, as oligonucleotides are unable to cross the blood-brain barrier. One possible method of delivery is through a pump inserted in the chest and connected to the brain by a catheter. In 2007, a nonprofit dedicated to finding a cure for HD donated 9.9 million dollars toward developing an antisense drug for HD. To date, antisense gene therapy has been successfully used to inhibit huntingtin production in cells, and is currently being tested in transgenic mice.

### G. Striatal Neuron Transplant

Striatal tissue graft or dissociated striatal suspension has also been suggested to hold promise as a therapeutic intervention in HD. The rationale for neural transplantation arises from the fact that neurodegeneration eliminates specific

neuronal populations, which can theoretically be replaced with the addition of new neurons, akin to organ transplant. In this system, it is proposed that transplanted neuronal tissue would reestablish the anatomical and functional aspects of the damaged and lost neurons (Dunnett and Rosser, 2007). Several preclinical and clinical studies in PD have provided proof-of-principle data suggesting the potential benefit of transplantation in the treatment of PD (Brundin *et al.*, 1986; Perlow *et al.*, 1979).

Initial studies using rodent chemical lesion models of HD have demonstrated successful striatal transplant survival, including dopamine terminal innervation of the transplant, and a recovery of striatal choline acetyltransferase and glutamic acid decarboxylase (Schmidt *et al.*, 1981). Subsequent studies have demonstrated functional recovery of motor behaviors after striatal transplantation (Deckel *et al.*, 1983). Similar studies in a nonhuman primate chemical lesion model of HD have demonstrated successful stereotaxic implantation of cross-species striatal neuronal grafts (rat to baboon) into the caudate-putamen (Isacson *et al.*, 1986). Post-transplant analyses revealed graft survival, with expression of striatal markers evident. Additional studies in nonhuman primates confirmed that grafting of striatal tissue into lesioned caudate ameliorated motor and behavioral alterations, demonstrating improved functional capacity (Schumacher *et al.*, 1992).

Using recommendations for trial criteria from the Core Assessment Program for Intracerebral Transplantation in HD (Quinn *et al.*, 1996), an initial pilot grafting paradigm was employed where three HD patients received bilateral transplantation of fetal striatal tissue into the caudate and putamen (Kopyov *et al.*, 1998). Graft survival was determined through comparison of pre and 1-year postsurgical MRI, with marked improvement in signal, consistent with graft survival. In all three patients, striatal tissue transplantation resulted in improved motor behavior as assessed by the UHDRS. In a subsequent safety and tolerability trial employing fetal striatal tissue transplants into the caudate and putamen (Bachoud-Levi *et al.*, 2000), several HD patients showed marked improvement in UHDRS scores. However, complications in the use of immunosuppressant therapies postoperatively were observed, making analyses impossible. Additional trials with cross-species striatal transplantation were performed using porcine fetal tissues in human HD patients (Fink *et al.*, 2000). Even with therapy to suppress immunological xenograft rejection, no surviving striatal transplants were observed, and no functional improvements noted. Together, these latter trials represent the difficulties of treating human HD with clinical striatal transplants.

While completed clinical trials demonstrate safety and tolerability, with adequate surgical procedures to perform the tissue transplants, certain aspects of tissue or cell preparation and delivery for surgical implantation remain unresolved. Furthermore, given ethical concerns regarding the use of fetal tissue, or the use of alternative cells such as porcine fetal grafts, issues arise regarding immunological function and management of tissue rejections (Barker and Widner, 2004). Finally,

while striatal cell transplant may hold promise for the treatment of HD, one caveat that remains is the significant gross neuropathology observed as disease progresses, with extra-striatal neuropathology present. Indeed, cortical neuropathology in HD likely contributes to many of the behavioral and cognitive disruptions associated with advanced disease. Since previous studies have not examined behavioral and motor performance beyond 1 year postoperatively, it remains to be seen whether striatal transplantation will have a long term, broad therapeutic benefit.

### H. Immunization

Immunization using vaccines has been moderately successful in animal models of Alzheimer's disease and ALS, but it is not clear whether this approach will be applicable to HD. DNA vaccination against toxic intracellularproteins conferred some therapeutic benefit on R6/2mice129 and ameliorated the diabetic phenotype of this mouse model of HD. Specifically targeting intracellular and particularly intranuclear huntingtin is quite adaunting challenge. The likelihood of the occurrence of serious side effects, like those recently reported in a Phase II clinical trial for Alzheimer's disease (Greenberg *et al.*, 2003; Nicoll *et al.*, 2003), which included encephalitis, must be considered before progressing with this therapeutic regimen.

## VI. Conclusion

The search for effective strategies aimed at halting or reversing the neurodegenerative process in HD will require extensive preclinical and clinical validation to provide the necessary safety and tolerability data for effective clinical use. While many of the compounds in this review have demonstrated significant potential in preclinical trials involving mice, it will take substantial effort to determine whether they are efficacious in HD subjects. The development of genetic models has greatly expanded our understanding of HD pathogenesis. These models also provide complex, yet accessible, biological systems with which candidate therapeutic compounds can be tested for efficacy and mode of action. While such models greatly enhance the discovery potential, it is important to understand the difficulty inherent in predicting the transference of success from mouse to man. Interestingly, recent evidence shows a parallel in efficacy in both HD patients and murine models of HD using antioxidant therapies in reducing peripheral oxidative stress levels of 8OH2'dG (Hersch *et al.*, 2006; Smith *et al.*, 2006). Early detection of disease will be of great importance in HD. Correlation of disease

biomarkers in both mouse and man will provide a powerful means to assess therapeutic treatments to humans experiencing HD and to predict the potential magnitude of benefits in these patients. Difficulties notwithstanding, preclinical therapeutic trials with murine models provide perhaps the best foundation on which to base human clinical trials. Importantly, data from preclinical trials using multiple models is likely to be most informative when assessing potential benefit in human HD.

## Acknowledgments

This work was supported by National Institutes of Health Grants NS045806, NS058793, and NS066912, and the Veterans Administration.

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# CELL-BASED TREATMENTS FOR HUNTINGTON'S DISEASE

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In experimental rats, mice, and monkeys, transplantation of embryonic striatal cells into the striatum can repair the damage and alleviate the functional deficits caused by striatal lesions. Such strategies have been translated to striatal repair by cell transplantation in small numbers of patients with progressive genetic striatal degeneration in Huntington's disease. In spite of some encouraging preliminary data, the clinical results are to date neither as reliable nor as compelling as the broad extend of recovery observed in the animal models across motor, cognitive, and skill and habit learning domains. Strategies to achieve immediate and long-term improvements in the clinical applications include identifying and limiting the causes of complications, standardization and quality control of preparation and delivery, appropriate patient selection to match the cellular repair to specific profiles of cell loss and degeneration in individual patients and different neurodegenerative diseases, and improving the availability of alternative sources of donor cells and tissues.

## I. Introduction

Following the success of the first clinical trials of cell transplantation in Parkinson's disease (Dunnett and Rosser, 2007; Lindvall *et al.*, 1990), trials have been extended to apply a similar strategy in other neurodegenerative disorders,

INTERNATIONAL REVIEW OF
NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00017-1




0074-7742/10 $35.00

such as Huntington's disease (HD) (Rosser and Dunnett, 2007), multiple sclerosis (Uccelli and Mancardi, 2010), amyotrophic lateral sclerosis (Mazzini *et al.*, 2010), spinal cord injury (Anderson, 2002; Ramón-Cueto and Muñoz-Quiles, 2011) and stroke (Kondziolka *et al.*, 2000). Of these, transplantation in HD has proved particularly informative about the potential of cell transplantation for alleviating both simple and complex motor deficits. The present review addresses the experimental foundations for cell transplantation in HD; the empirical extent (and limitations) of functional recovery; and the mechanisms by which grafts exert their functional effects. We highlight how a translational approach has revealed unanticipated consequences of restoring neural substrates of motor learning, first discovered as theoretical principles in experimental animals, which inform the practical day-to-day management of rehabilitation in patients.

The basic methods and principles that determine effective engraftment turn out to be rather similar, whatever the disease application. First and foremost, the state of plasticity of the cells at the time of transplantation is critical. To survive the transplantation process and to integrate into the host brain, neurons need to be implanted when they are immature; they are about to or have just undergone final cell division, their fate is determined, and they are entering an active growth phase. At the present stage of neural transplantation technology, this condition is most readily met by harvesting embryonic neurons for grafting during a precise window of ontogenetic development at the stage of their final mitosis (Dunnett and Björklund, 2000b; Olson *et al.*, 1983), which may be determined from anatomical studies of development (with birth dating using $^{3}$H-thymidine or bromodeoxyuridine), and validated by empirical studies of survival and phenotypic differentiation either following dissociated cell culture *in vitro* or following transplantation *in vivo*. At that stage in development, the endogenously encoded growth program allows newly born neurons to undergo final phenotypic differentiation, extend neurites, and establish reciprocal connections with cells of the host environment. Alternative sources of pluripotent cells with a similar capacity for survival, neuronal differentiation, and integration following transplantation are an active topic of current research, predominantly from embryonic (ES) and induced pluripotential (iPS) stem cell sources (Kim and De Vellis, 2009; Koch *et al.*, 2009). However, these alternatives have not yet reached systematic functional evaluation and the present review of the impact of cells on motor capacity in HD will focus on the well-established protocols for allografting fetal donor cells.

A second general feature required for effective cell transplantation is that the cells must be placed into a suitable environment. On the one hand, this involves the requirement that the host tissue environment has an appropriate cellular and microvascular composition that can support and sustain the implanted cells (Dunnett and Björklund, 2000a; Stenevi *et al.*, 1976), in particular in the critical period of several days following transplantation until the grafted cells are fully incorporated into the host microenvironment. The implanted neurons will be

dependent upon the trophic and glial environment of the host for their survival, terminal differentiation, and the stimulation and direction of neurite outgrowth. Embryonic neurons target neurite outgrowth to appropriate and specific denervated proximal targets; long-distance outgrowth is critically dependent upon appropriate guidance substrates although the capacity for long-distance axon growth from embryonic neurons in the adult brain (Björklund *et al.*, 1976; Thompson *et al.*, 2009; Wictorin *et al.*, 1990) is clearly greater than the classical perception that endogenous axon regeneration in the adult mammalian CNS is invariably abortive (Cajal, 1928).

The third concern of all transplantation programs, that grafts will be rejected by the host immune system, is less critical where allografts of fetal neurons in the brain are generally well-tolerated immunologically, by virtue both of their low immunogenicity and of the relative immunological privilege of the host CNS environment (Lund and Bannerjee, 1992; Widner, 1998). An ongoing low-grade immunological response has been reported in several postmortem cases of neuronal transplantation in both PD and HD, in terms of infiltration of microglia and inflammatory cells (Capetian *et al.*, 2009; Keene *et al.*, 2007; Mendez *et al.*, 2005), although such responses are typically insufficient to lead to outright rejection. Since the issues of allograft response in human brain remains poorly understood, prophylactic immune suppression (typically with cyclosporine, prednisolone, and azathioprine, in various combinations) is typically administered for 6–24 months in most clinical transplantation programmes (Bachoud-Lévi *et al.*, 2000a; Hauser *et al.*, 2002; Kopyov *et al.*, 1998; Rosser *et al.*, 2002), although others argue that evidence for the necessity of such treatments remain lacking (Freed *et al.*, 2003).

## II. Present Status

### A. Graft-Derived Recovery in Simple Motor (and Cognitive) Tasks in Animals

Injections of excitotoxic amino acids (such as kainic, ibotenic, or quinolinic acids) into the striatum of rats induce selective degeneration of intrinsic striatal neurons (in particular the medium spiny projection neurons, MSNs), along with marked striatal atrophy and expansion of the lateral ventricles, whilst sparing intrinsic glial cells and myelinated axons of the internal capsule (Coyle and Schwarcz, 1976; Schwarcz *et al.*, 1979, 1983). This profile mimics the core neuropathology of HD, and provided the first viable experimental model of the disease in animals. Moreover, rats, mice, or monkeys with excitotoxic striatal lesions exhibit marked behavioral deficits in both motor and cognitive domains, again reflecting the functional pathology of the human disease (Sanberg and Coyle,

1984). Embryonic striatal neurons implanted into the lesioned striatum survive (Isacson *et al.*, 1986; Schmidt *et al.*, 1981), differentiate to replace all major neuronal types of the striatum within the grafts (Clarke *et al.*, 1994; Helm *et al.*, 1990), restore many of the neuronal neurochemical markers of the normal striatum (Isacson *et al.*, 1985), and establish basic afferent and efferent connections with the host brain (Wictorin, 1992). Thus, grafted cells stimulate sprouting and innervation from relevant populations of host axons that have lost their normal striatal targets, and axons of the grafted neurons have the capacity to reinnervate appropriate targets in the host brain (Wictorin, 1992) (see Fig. 1). Both in-growing host axons and out-

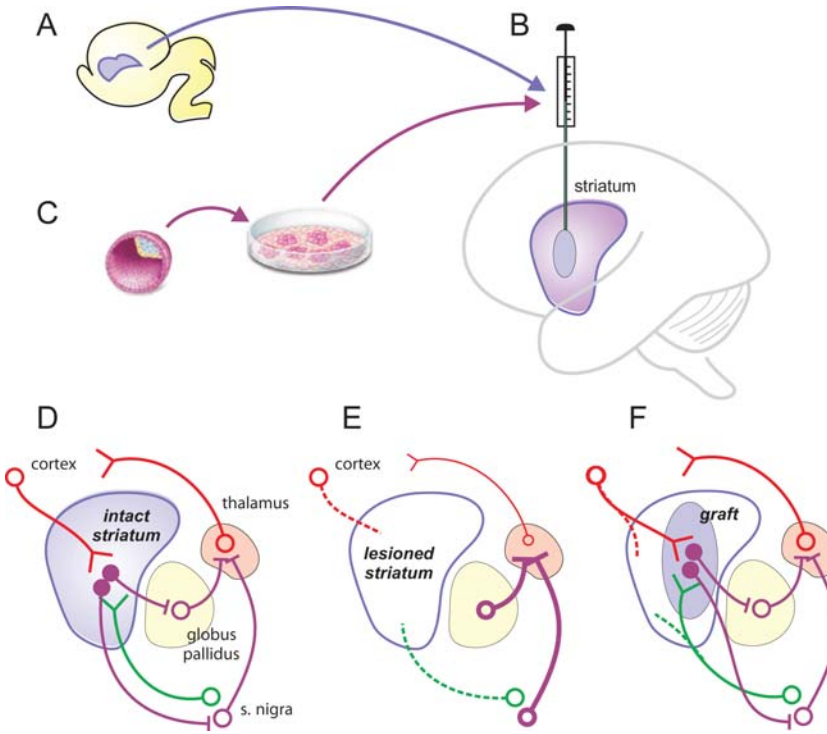


FIG. 1. Schematic illustration of graft derived reconstruction of the neostriatum following striatal lesions in experimental animals or in Huntington's disease in man. (A) Striatal dissection of ganglionic eminence in fetal brain. (B) Implantation into adult host striatum. (C) Alternative sources of cells derived from neuronal differentiation of embryonic stem cells. (D) Basic corticostriatal circuitry of the normal mammalian brain, indicating excitatory glutamatergic projections (red), inhibitory GABAergic connections (purple) and the regulatory nigrostriatal dopaminergic projection (green). (E) Loss of GABAergic medium spiny projection neurons following striatal lesion or in HD results in complete disconnection of corticostriatal circuits. (F) Reconstruction of corticostriatal circuitry by striatal grafts. See the text for details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.).

growing graft-derived axons are seen to establish morphologically appropriate synaptic connections with their appropriate targets in the grafts and host brain, respectively (Clarke and Dunnett, 1993). Thus, grafted neurons have the capacity to reconstruct the cardinal features of the damaged corticostriatal loop circuitry, at least qualitatively – the essential definition of "brain repair" (Dunnett, 1995). However, is the local organization, with or without intrinsic plasticity in the newly forming connections, sufficient to impact on host function to yield functional recovery alongside the structural repair?

Yes. Early studies used animals with bilateral lesions, the most obvious feature of which is locomotor hyperactivity. Striatal grafts alleviate the hyperactivity syndrome, in particular during the nocturnal period and under food deprivation when the levels of arousal are most marked (Deckel *et al.*, 1986; Giordano *et al.*, 1990; Isacson *et al.*, 1986). These first studies also showed that deficits in simple cognitive tests of spatial learning and passive avoidance memory, characteristic of the "fronto-striatal" impairment of striatal lesioned animals, were also alleviated by the grafts (Deckel *et al.*, 1986; Isacson *et al.*, 1986; Piña *et al.*, 1994). More detailed analysis of the motor deficits have been undertaken in unilateral lesioned rats, in which spontaneous and drug induced rotation, contralateral neglect, turning biases in choice responding, and impairments in skilled forepaw use in reaching tasks, are all significantly alleviated by the transplants (Dunnett *et al.*, 1988b; Fricker *et al.*, 1997; Kendall *et al.*, 1998; Mayer *et al.*, 1992; Montoya *et al.*, 1990). All these results indicate that striatal grafts can provide an effective recovery of both simple motor deficits and more complex features of motor selection and action in animals with striatal lesions that mimic the primary neuropathology of human HD.

Nevertheless, excitotoxic lesions do not reproduce the core pathogenic process of HD which is of essence a slowly progressive autosomal dominant genetic disease arising from a single gene mutation involving a CAG triplet expansion in the huntingtin gene, located on the distal arm of chromosome 4 (Huntington's Disease Collaborative Research Group, 1993). Transgenic and knock-in models are now well established that reproduce this single gene mutation in mice (Heng *et al.*, 2008; Mangiarini *et al.*, 1996), and have revealed important features of the pathogenic process in HD, notably the affinity of fragments of mutant huntingtin to aggregate and form protein inclusions in the nuclei and cytoplasm of affected neurons (Davies *et al.*, 1997). Although the different transgenic lines have proved valuable in evaluating a range of pharmaceutical and neuroprotective strategies to inhibit inclusion formation, slow cell death, and prolong life (Chen *et al.*, 2000; Ferrante, 2009; Karpuj *et al.*, 2002; Ramaswamy *et al.*, 2009; Rose *et al.*, 2010; Wang *et al.*, 2010), they have so far proved less informative in evaluating the functional efficacy of cell therapies. A major reason for this is that the inclusion pathology in most transgenic lines which exhibit rapidly progressing motor and cognitive phenotypes is very widespread (much more so than seen in the vast majority of HD

postmortem cases), affecting all areas of brain, and not restricted to the striatum as would be required to actually test a striatal repair strategy. Consequently, although transplanted striatal cells have been seen to survive in the HD transgenic brain, and exhibit minor benefits at the time of onset of motor pathology, they do not halt the widespread progression of disease or the associated multi-system syndrome and early death (Dunnett *et al.*, 1998). The more recent development of knock-in lines that exhibit more focal striatal pathology are only now being characterized behaviorally, and the associated motor syndrome is typically mild, slowly progressing, and only detectable relatively late in a life span (Brooks *et al.*, 2011a, 2011b). As a result, none of these models have yet been used for systematic evaluation of novel therapeutics whether neuroprotective or reconstructive. With the presently available tools, the excitotoxic lesion remains the best model for systematic evaluation of the transplantation technologies for striatal repair; and their relevance for clinical application can ultimately only be evaluated in well designed trials in patients (see Section II C).

### B. Graft-Derived Recovery and Circuit Reconstruction

A notable feature of striatal grafts in the striatal lesioned brain is that they appear capable of establishing both afferent and efferent connections with the host brain, replacing an essential component in the corticostriatal circuitry. However, just because grafted cells appear anatomically to reconstruct a damaged circuit does not necessarily mean that that is the basis or mechanisms of the functional recovery (Dunnett, 1995; Dunnett *et al.*, 2000). Indeed, for nigral grafts in animal models of PD, the grafts do provide a dopaminergic reinnervation of the dopamine-depleted striatum, but they must be placed into the ectopic striatal target sites directly in order to do so (Björklund *et al.*, 1987). A nigral graft placed into the area of intrinsic cell loss in the host substantia nigra, showing very little capacity for long-distance axon growth back to the striatum, certainly does not reconstruct the damaged circuits and has very limited functional impact on the motor capacity of the host (Björklund *et al.*, 1983; Dunnett *et al.*, 1983). Similarly, adrenal medulla grafts implanted into the dopamine-depleted striatum of parkinsonian rats, turn out to largely influence function not by local secretion of the deficient endogenous neurotransmitter, as first thought, but by secreting a range of trophic molecules (most likely including GDNF and BDNF) that stimulated sprouting of host dopamine terminals spared by making partial lesions (Bohn *et al.*, 1987). Thus, it is now apparent that grafts can theoretically, and do in practice, exert functional influence over host behavior (including, but more than, recovery of function after lesion) by a variety of mechanisms (Björklund *et al.*, 1987; Dunnett, 2009) that include

(i) *nonspecific:* effects of surgery rather than of the cell replacement *per se*;
(ii) *neuroprotective*: release of factors that slow lesion or disease progression;

(iii) *trophic*: release of factors that stimulate host plasticity, compensation and sprouting;
(iv) *target support*: provision of alternative targets of target deprived axons;
(v) *bridges*: provision of glial substrates for host axon regeneration;
(vi) *pharmacological*: replacement of deficient neurochemicals and neurotransmitters;
(vii) *reinnervation*: tonic local reinnervation allowing target reactivation;
(viii) *circuit reconstruction*: afferent and efferent integration of grafted cells into host neuronal circuit;
(ix) *full repair*: complete and appropriate reorganization of grafted cells to restore an intact, appropriately regulated, and fully functional host neuronal network.

What has become apparent over the last two decades is that there is not a single answer to the question "how do grafts work?" Different cells can influence behavior by quite different mechanisms even in the same model. For example, in the Parkinson's disease model rat, mechanisms (i), (ii), (iii), (v), (vi), and (vii) have all been evidenced with different cell types and surgical approaches (Björklund *et al.*, 1987; Dunnett and Björklund, 2010).

Moreover, different behavioral tests or classes of symptom may require different levels of repair and mechanisms of recovery to alleviate the deficits and restore function. This is well illustrated for the comparison between rotation and skilled paw reaching in animals with unilateral dopamine or striatal lesions. First, the graft must be appropriate to the lesion: only nigral grafts implanted into the striatum will restore motor asymmetries in dopamine depleted rats, whereas only striatal grafts implanted into the same striatal site will restore deficits in striatal lesioned rats (Dunnett *et al.*, 1988a; Montoya *et al.*, 1990). Second, nigral grafts (in PD rats) will alleviate unilateral deficits in rotation and neglect but not the impairments in skilled paw reaching (Dunnett *et al.*, 1987b), whereas striatal grafts (in HD rats) are extremely proficient in also restoring skilled reaching with the contralateral paw (Dunnett *et al.*, 1988b; Montoya *et al.*, 1990). We have hypothesized that the major difference between the two models is that nigral grafts are ectopic, restoring dopamine activation at striatal targets but not reconstructing the lost nigrostriatal pathway, whereas striatal grafts are homotopic, replacing the lost striatal cells and observed to actually reconstruct the disconnected corticostriatal circuitry (Dunnett, 1995; Dunnett *et al.*, 1987b; Montoya *et al.*, 1990). Indeed, although circuit reconstruction is rarely sustainable as the leading hypothesis for the mechanism of recovery in most transplantation models of disease, we have argued that the best evidence where reconstruction at this level of repair is actually achieved is for striatal repair by striatal grafts in this animal model of HD (Dunnett, 1995). A number of lines of evidence support this conclusion. Firstly, striatal lesions induce clear cognitive deficits as well as motor deficits, and the profile of deficits reflects

the topography of corticostriatal projections, such that the consequences of a striatal lesion are to "disconnect" the essential corticostriatal pathway ("loop") involved in the translation of plans into actions (Divac *et al.*, 1967; Dunnett *et al.*, 2005). An essential feature of such functional systems is that performance is disrupted by lesions in any component of the circuit, including the fiber connections as well as intrinsic processing nuclei (Rosvold, 1972). More relevant to the present argument, function can only be restored by re-establishing the damaged circuit. Striatal grafts do indeed restore performance on prototypical frontal cognitive tasks such as delayed spatial alternation, both in mazes (Isacson *et al.*, 1986) and in operant lever pressing tasks (Dunnett and White, 2006). Not only do such striatal grafts replace lost MSNs where they belong in the circuit, but the ultrastructural studies alluded to in Section II A include the demonstration that host cortical axons synapse onto the heads of spines of grafted MSNs that project onward to the host globus pallidus, and which receive intrinsic regulation from host dopaminergic inputs making synaptic contacts onto the necks of the spines of the same grafted MSNs (Clarke and Dunnett, 1993; Clarke *et al.*, 1988). Thus, functional recovery is seen in animals in which the essential components of the corticostriato-pallidal circuit under dopaminergic regulation is reconstructed on complex tasks which specific disconnection lesions have shown to be dependent upon the integrity of that of the frontal cortical circuit. In addition, both electrophysiological (Rutherford *et al.*, 1987; Wilson *et al.*, 1990; Xu *et al.*, 1991) and *in vivo* neurochemical (Campbell *et al.*, 1993; Sirinathsinghji *et al.*, 1988, 1993) recordings have demonstrated functional graft–host and host–graft signalling. Nevertheless, in view of the reduced density of connections, local collateral signaling, and feedback may be less than that observed in the normal striatum (Xu *et al.*, 1991).

### C. Graft-Derived Recovery in HD Patients

Eight centers have now reported on the feasibility and safety of striatal cell transplantation in HD in 29 patients (Bachoud-Lévi *et al.*, 2000b; Gallina *et al.*, 2008; Hauser *et al.*, 2002; Kopyov *et al.*, 1998; Madrazo *et al.*, 1993; Reuter *et al.*, 2008; Rosser *et al.*, 2002; Sramka *et al.*, 1992), and there are approximately 70–80 further patients known to have received grafts but not yet reported in published studies (Freeman *et al.*, 2011; Rosser *et al.*, 2011). The most compelling evidence that the grafts can not only survive but alleviate some of the functional impairments of the human disease is provided by a series of reports on the first five patients from the French series coordinated by Marc Peschanski and Anne-Cathérine Bachoud-Lévi (2000b; Bachoud-Lévi *et al.*, 2000a, 2006; 2009). Three of the five patients exhibited surviving grafts in the functional imaging and a stabilization of the UHDRS motor scale was noted in the same three patients; a fourth showed temporary improvement

associated with a surviving graft, but following an acute episode where the graft appeared to be lost the recovery also relapsed; and the fifth patient showed no clear surviving grafts from the outset and never exhibited any functional improvement. Correlating with both imaging and neurological data, the three patients seen to benefit from the graft also exhibited objective improvements in electrophysiological measurements of somatosensory evoked potentials and in video tracking of sequence tapping (Bachoud-Lévi *et al.*, 2000b). These same patients were seen to show a stabilization of benefit over a period of up to 6 years following transplantation (Bachoud-Lévi *et al.*, 2006).

In other centers, a similar marked benefit has been documented in one of two patients operated at Kings College London, and striatal like tissue was seen to survive in the graft in [$^{11}$C]-raclopride PET imaging in this patient (Reuter *et al.*, 2008). There have also been clinical reports of functional improvement in both neurological and neuropsychological measures in several patients from the Los Angeles series (Kurth *et al.*, 1996; Philpott *et al.*, 1997). Conversely, in a series of seven patients operated at the University of Florida, six appeared to show improvement over the first year, but one patient deteriorated markedly so that the change in comparison to baseline was overall not significant (Hauser *et al.*, 2002) and was accompanied by marked surgical complications involving subdural hematomas in three patients. In our own series of five patients, operated in Cambridge, striatal cell transplantation has been found to be safe (Rosser *et al.*, 2002) but the completion of the series has been temporarily suspended pending achieving compliance with revised European regulations for medicinal grade processing of tissues for human application (European Parliament and Council, 2004). Finally, a recent report from Italy suggests detectable short-term alleviation of some motor scores (Gallina *et al.*, 2010), but with significant expansion of the grafts that may suggest an atypical overgrowth of the transplants (see Section D, below).

Thus, to summarize, we now have clear data that striatal cell transplantation in HD is feasible and that the grafts can survive transplantation. Recovery was seen in some but not all patients in several centers, and functional benefit appeared to be contingent upon survival of the grafts as determined by MRI and/or PET imaging (Freeman *et al.*, 2011; Rosser *et al.*, 2011). Conversely, there have been several reports of side effects that may complement our views on the safety and long-term stability of (at least some of) the present protocols.

### D. Limitations and Complications after Striatal Cell Transplantation

The complications that have arisen in the first clinical trials fall into four main categories of concern.

Firstly, one center has reported subdural hematomas in a total of three HD patients (Hauser *et al.*, 2002), a problem which was notably absent in nigral

transplantation surgeries in PD from the same centre (Olanow *et al.*, 2003), although other side effects involving the emergence of graft-induced dyskinesias in several patients was noted in that study. A survey of the patients operated in the different centers suggests that the HD patients selected for this trial were at a relatively advanced stage of disease in comparison to patients selected for operation elsewhere. The greater cortical atrophy that is expected in more advanced patients is a clear risk factor for surgical bleeds, and these observations caution that more advanced disease involving extensive cortical atrophy should be a clear exclusion factor in future trials. Particular attention will need to be paid to surgical routes of approach determined from surgical MRI that allow clear traverse of the cortical gyri with the injection needles, without risk of damaging the walls of the cortical sulci.

Secondly, whereas several post mortem studies have now reported appropriate differentiation of human striatal grafts into discrete striatal-like zones rich in DARPP-32 positive neurons (Capetian *et al.*, 2009; Cicchetti *et al.*, 2009; Freeman *et al.*, 2000; Keene *et al.*, 2007), there have now been at least two occasions of marked tissue overgrowth in individual HD patients (Gallina *et al.*, 2010; Keene *et al.*, 2009). This factor was a concern in the design of early trials since individual rats bearing human fetal striatal xenografts were seen to exhibit significant graft expansion so as to cause space occupying lesions in the host brain (Aubry *et al.*, 2008; Brundin *et al.*, 1996). With further experience in the xenografts models it has generally been concluded that xenografts overgrowth primarily reflects the very growth capacity of human striatal tissue in the much smaller rat brain; no similar overgrowth has been seen in rat-to-rat, mouse-to-mouse, or primate-to-primate allografts, and so this concern about human striatal allografts in man has generally waned. In the first clear case of tissue overgrowth in the clinical application of striatal transplantation, an HD patient came to post mortem with marked cyst formation and large expansion of non-neuronal tissues within the graft mass (Keene *et al.*, 2009); this clearly suggested that the graft comprised epithelial tissues as well as fetal brain and suggested mis-dissection of the donor tissues taken for implantation (Freeman *et al.*, 1999; 2010). More recently, several patients in a recent series have exhibited massive expansion of the graft tissue, when visualized in MRI, from the striatal site of implantation both back along the trajectory of implantation into the overlying neocortex and more ventrally into the ventral striatum (Gallina *et al.*, 2010). As yet, these patients have shown no specific adverse effects, and the authors themselves view these grafts as a positive sign of migration, differentiation and integration. However, the observations have been considered by others to exhibit worrying features of overgrowth, and potentially to reflect a similar mis-dissection to include non-neuronal tissues (Freeman *et al.*, 2011), requiring careful monitoring and follow-up. Together these observations do not change our view that implantation of embryonic striatal tissues can be as safe in man as in animals, but they do indicate that particular attention needs to be paid to

experience, training, and quality control (including maintaining archives of all tissues implanted clinically) to ensure that we know exactly what tissue is being implanted. In the light of the intrinsic variability of quality of fetal tissues derived from elective abortions, especially when derived from a surgical aspiration procedure, so maintaining an accurate and reproducible tissue dissection will always prove difficult. However, improved standardization may be achieved using fetal tissues derived from better preserved medical terminations (Kelly *et al.*, 2011), and even more so when using long-term expanded cell lines derived from pluripotent ES and iPS sources which have far better prospect for extensive quality control *in vitro* prior to use.

Thirdly, the presence of an ongoing immune response, even in the presence of immunoprotective 'triple' therapy, cannot be excluded. One patient in the first French series has shown loss of graft and functional relapse following an apparent infection, suggestive of graft rejection, and 4 of 13 patients in the second series have exhibited circulating antibodies indicative of alloimmunization against the grafts although not at a level to precipitate acute rejection or loss of the grafts (Krystkowiak *et al.*, 2007). Similarly, in several post mortem cases from the Florida series, detailed histochemical analysis has indicated the presence of a low level inflammatory response and invasion of microglia at the transplantation site (Cicchetti *et al.*, 2009). Similar responses have been seen to nigral grafts in the PD brain up to 18 years following transplantation (Kordower *et al.*, 1997; Olanow *et al.*, 2003). The functional significance of such on-going low-level immunological responses effects is not understood – it appears to be insufficient to lead to graft rejection even over an extended time period, but may nevertheless lead to some long-term compromise of graft function, and cannot be ignored until better understood.

Finally, whereas neurological and neuropsychological changes appear to improve, if they change at all, there has been the suggestion from the French series that grafted patients may nevertheless exhibit an exacerbation of psychiatric signs. This has not been systematically evaluated, and psychiatric problems are a major feature of disease progression in HD, but several of these patients were considered to exhibit an increase in irritability and emotional lability, and may have contributed to difficulties across the group in maintaining stability in taking their immunoprotective drugs.

### E. Motor Learning and Behavioral Plasticity in Animals and Man

One feature of the preclinical experimental studies may bear important relevance to optimization of the design and conduct of the clinical trials, namely that training and experience can markedly enhance the functional efficacy of the grafts. This realization arose from studies designed initially to assess the effects of striatal

grafts in more complex forms of motor learning and habit formation in rats, over and above their demonstrated efficacy on direct motor control.

We have used a "9-hole box" operant test apparatus to train rats to make rapid lateralized responses to briefly presented light stimuli occurring in a horizontal array of holes, similar to touch screen vigilance and reaction time tests in man (Fig. 2). Striatal lesions disrupt the speed and accuracy of responding to stimuli presented on the side contralateral to the lesion (Brasted *et al.*, 1997; Mittleman

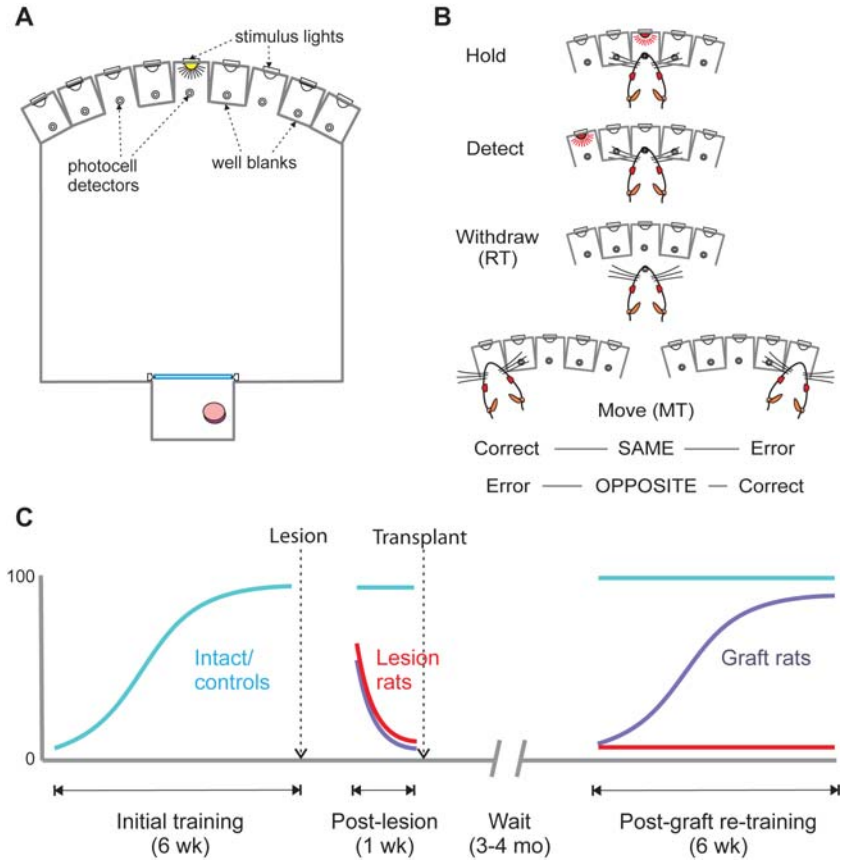


FIG. 2. (A) Schematic illustration of the 9-hole box operant chamber. (B) Illustration of the presentation of lateralized stimuli to which the rat must attend and respond in the choice lateralized reaction time task. In the "SAME version, a response in the hole on the same side of the stimulus light is rewarded, and a response in the opposite hole punished, and vice versa for the "OPPOSITE" version of the task. (C) Schematic illustration of task acquisition, disruption of performance following lesion, and relearning of accurate task performance following striatal transplantation that underlies the "learning to use the transplant" hypothesis. See the text for details. (For color version of this figure, the reader is referred to the web version of this book.).

*et al.*, 1988), a deficit which can be almost completely alleviated following implantation of striatal grafts (Brasted *et al.*, 1999a, 1999b, 2000; Döbrössy and Dunnett, 1998; Mayer *et al.*, 1992). However, the time frame of recovery is informative. Normal rats require approximately 6 weeks of daily training to learn accurate lateralized responding to an asymptotic level of performance. Following lesion, performance is immediately and profoundly disrupted on the side of the body contralateral to the lesion, whereas they continue to respond rapidly and accurately on the ipsilateral side (where response selection is under the control of the intact striatum). Animals are typically left for 3–4 months following transplantation, to allow full growth of the implanted striatal cells and their integration into the host neuronal circuitry, before testing. First the grafted animals are as deficient as rats with long-term lesions, but they then relearn to perform rapidly and accurately on the contralateral side also, over a similar period of 6–8 weeks as required to learn the task *de novo* (Brasted *et al.*, 1999a, 1999b, 2000). The grafted cells are not simply enabling the rats to perform the motor response *per se*; rather, these results clearly suggest that the grafted cells are restoring the essential neuronal substrate for new motor learning *de novo*, a phenomenon which we have described as "learning to use the transplant" (Döbrössy and Dunnett, 2001; Mayer *et al.*, 1992).

At the theoretical level, this phenomenon suggests not only that the grafted striatal cells repair the normal corticostriatal circuitry, but reconstruct the normal striatal substrate for learning motor skills and habits (Döbrössy and Dunnett, 2001). Thus, we have used a transfer of training paradigm to provide further direct evidence that relearning the specific stimulus-response associations underlying lateralized reaction time performance takes place within the grafted striatal circuits (Brasted *et al.*, 1999a). More recently, we have used electrophysiological recording in tissue slices to demonstrate that the normal plasticity in the form of long-term depression at the direct corticostriatal synapse is reproduced under similar conditions at the reformed synapses made between afferent corticostriatal axons and the spines of grafted MSNs (Mazzocchi-Jones *et al.*, 2009).

At the applied level these data indicate that functional recovery is not simply dependent upon the anatomical reconstruction achieved by the grafted cells. In addition, the animal (or patient) must relearn the skills and habits previously acquired through a lifetime of experience but lost through the lesion or disease using the graft-derived replacement striatal circuits (Döbrössy and Dunnett, 2001). In experimental animals, we have used forced exercise and different forms of reaching tests to demonstrate that the transfer of training is relatively task-specific (Döbrössy and Dunnett, 2003, 2005), and is associated with specific structural as well as physiological changes, for example in spine density of MSNs within the grafts (Döbrössy and Dunnett, 2004, 2006, 2008). In the clinical context, these data strongly suggest that the fate of HD patients in transplantation programs will be markedly enhanced if combined not simply with neurological and neuropsychological assessment, but also with the provision of a systematic physiotherapy

program of training in a broad range of motor skills and habits relevant to the activities of daily living (Döbrössy and Dunnett, 2001; Döbrössy *et al.*, 2010). Such training programs have recently been shown to be beneficial for normal patients (Busse *et al.*, 2008; Busse and Rosser, 2007), and may be expected to be doubly valuable following a reconstructive surgical cell therapy.

## III. Future Developments

### A. Improvements in Striatal Repair

We conclude from the preceding description that striatal grafts can work well to reconstruct the damage and alleviate a range of motor and cognitive deficits in experimental animals with specific striatal lesions, and that these results provide preliminary encouragement for development in clinical applications in HD. We consider that the major side effects and complications that have been reported can be eliminated by better patient selection and adoption of improved cell preparation and surgical protocols. However, the variable and incomplete recovery observed in most patient series raises further concerns, that may only partly be addressed by identifying improved sources of donor cells, by optimizing protocols for preparation of tissues and their surgical delivery; and by better patient selection and management. We also need to consider the extent to which extra-striatal features of the human disease limit the extent to which striatal repair can address major components of the disease syndrome, and seek to extend the profile of protection and reconstruction to other affected systems of the brain. Conversely, if not HD, are there other more focal striatal syndromes (such as specific forms of striatal infarct or ischemia), that might be more amenable to the transplantation approach to striatal reconstruction that has proved so effective in experimental animals?

First and foremost, the neural transplantation field, along with other areas of cell therapy, has undergone significant transformation in the last decade with the enactment of national and regional regulation requiring adoption of standard operating principles, quality control, and preparation under strict compliance with "good manufacturing practice" and sterile environment (European Parliament and Council, 2004). This will certainly ensure a degree of standardization in methods. In addition, an emphasis on documented training and validation of protocols has the prospect of reducing the incidence of poor dissection resulting in implantation of inappropriate non-neuronal tissues, whether or not associated with a marked capacity for proliferation and uncontrolled growth. Nevertheless, the rigors of operating to GMP can introduce constraints to protocols that are demonstrably suboptimal in terms of their biological efficacy simply to meet standards of compliance, for example aiming to use only using reagents that

are traceable, carry legal audit certification, and involve entirely synthetic or nonanimal derived products.

More problematic is the quality of supply of donor tissues. Tissue from surgical (aspiration) terminations of pregnancy is typically fragmented, making accurate identification of forebrain features and precise dissection extremely difficult to standardize, even with considerable experience. Determination of fetal age/stage of development when based on clinical records, ultrasound notes and morphometric measurement can be quite variable. Nor can such tissues ever even approach sterility, requiring systematic and thoroughly validated washing protocols with microbiological monitoring an essential quality requirement. Even with the use of a hibernation protocol to extend the period of tissue viability for 5–7 days from collection to transplantation (Hurelbrink *et al.*, 2003; Sauer and Brundin, 1991), the time to complete assays of maternal donor blood and tissue products for viral and microbiological contamination is limited, and some testing (such as for prion disease) is essentially excluded. Fetal tissues derived from medical terminations of pregnancy have recently been shown to have comparable viability for cell transplantation (Kelly *et al.*, 2011), and this source may provide significant improvements in the reliability of supply, the integrity of the fetal brain allowing more accurate dissection, and the relative cleanliness of donated tissues within the placental sack, but the variability of age, limited time window of utilization, and impossibility of standardization remain. For these reasons, attention is increasingly turning to pluripotent ES and iPS sources of cells for transplantation.

Stem cells are characterized by their capacity to be expanded for prolonged periods *in vitro*, and to subsequently differentiate (under appropriate conditions) into any cell type of the body (Zietlow *et al.*, 2008). In principle, validated stem cell lines offer the prospect of providing an unlimited supply of standardized and quality assured cells for transplantation, 'off the shelf' as and when required. There are a number of published protocols now available for the neuralization of ES-derived cells (Gaspard and Vanderhaeghen, 2010), and protocols are being developed for the differentiation of a number of specific neuronal phenotypes (Peljto and Wichterle, 2011). The phenotype for which most progress has been made at the time of writing, largely by attempts to recapitulate developmental signals, is probably the midbrain A9 dopaminergic neuron (Kriks and Studer, 2009; Pruszak and Isacson, 2009; Thomas, 2010). There are numerous reports of dopamine cells with properties consistent with an A9 dopamine phenotype *in vitro* although reports of the survival and differentiation of such cells following transplantation has been much less consistent. Preliminary protocols for derivation of striatal-like MSNs, using DARPP-32 positivity as a primary marker, are also beginning to appear (Aubry *et al.*, 2008). However, at present the optimal protocols are yet to be determined, and yield a mixed variety of cell types rather than reliably and exclusively developing neurons of the specific target phenotype. As for the dopamine neurons, the survival and stability of a differentiated phenotype

following CNS transplantation has been far less reliable than is typically achieved using newly differentiated primary fetal neurons, and they have quite different capacities for migration, neurite outgrowth, physiological function, and connectivity with the host. In summary, although rapid progress is being made, our ability to control and direct differentiation of stem cells, whether of ES, iPS or adult somatic origin, remains incomplete and we remain a long way from being able to provide a replacement for developing fetal neurons with a similar capacity for phenotypic specificity, integration and reconstruction of damaged adult neuronal circuitry.

In addition, reflecting their capacity for expansion in an undifferentiated state, all stem cell sources have a capacity to form tumors. Notwithstanding active research both into ways to ensure complete *in vitro* differentiation into a nonproliferative state and alternative strategies such as the introduction of suicide genes for regulated elimination in case of adverse overgrowth, the outstanding concerns for safety have yet to be fully resolved. Thus, although they offer a compelling opportunity for resolution of the current limitations on availability of supply, standardization and quality control, no stem cell source is yet close to readiness for legitimate clinical application. Which is not to say that such clinical translation of stem cell programs are not already proceeding, rather that in our opinion they are seriously premature on grounds both of safety and of efficacy (for which neither theoretical nor empirical evidence is compelling).

### B. Patient Selection and Disease

As noted above, we also need to consider the extent to which extra-striatal features of HD influences the extent and limitation of recovery following striatal transplantation. Although HD has traditionally been considered a striatal disease, and striatal cell loss and atrophy are certainly the earliest appearing and most marked features of the neuropathology (Vonsattel *et al.*, 1985), as the disease progresses, the neocortex and other output nuclei of the basal ganglia, along with other brain nuclei such as hypothalamus, and peripheral organs also become progressively affected (Gutekunst *et al.*, 2002). While we remain unclear about the mechanisms whereby the genetic mutation that is expressed in all cells is translated into cellular dysfunction and cell death in some cell types and anatomical systems, but not in others, it remains a matter of dispute the extent to which the above temporal profile reflects either (i) an anatomical cascade of pathogenic influence originating in the striatum versus (ii) independent manifestations of the disease process in striatal, cortical, and other areas. Nevertheless, the answer to this issue will profoundly influence the potential long-term benefit that could be expected following an effective reparative striatal cell therapy. If (i), then alleviating the striatal degeneration by striatal cell replacement might provide effective

trophic and target support for cortical afferents and downstream basal ganglia efferents, halting the progressive spread of the disease. Conversely, if (ii), then no amount of striatal repair will alleviate the ongoing cortical decline which almost certainly (along with the striatal degeneration) contributes to the major cognitive and psychiatric features of the disease. In this circumstance, then whereas early striatal transplantation may provide an effective remission of symptoms, long-term benefits are likely to be limited. Certainly there are those that argue that the second possibility is the more likely and consequently, further trials of striatal transplantation should not continue (Albin, 2002). We believe that this is overly bleak, and while we do not know the causative relationship, cutting off one promising line of investigation might be considered foolhardy. On the other hand, throughout the course of the disease the striatal degeneration precedes and is more marked than cell loss and atrophy in other areas, and since striatal circuits as a core component of functional frontal systems are involved in cognitive and behavioral as well as motor symptoms we consider that effective striatal repair is likely to provide benefit across the functional domains, even in advanced disease. At the same time, we would not wish to argue that any therapeutic strategy should be pursued in isolation. Almost certainly, patients will best be served by judicious combination of symptomatic, neuroprotective and reparative therapies rather than rigid pursuit on theoretical grounds of one approach to the exclusion of others.

Nevertheless, a cautionary word is required against the assumption that if cell transplantation works for striatal repair, then why not adopt a similar approach for cortical, pallidal or other regions of degeneration also. As described above (Section II B), different grafts influence host behavior via a variety of different mechanisms, and there are different functional demands dependent upon the behavioral class and anatomical system transplanted. The striatum is uniquely suited to such circuit reconstruction by virtue of its anatomical location at the point of convergence of diverse cortical inputs and yet it retains into adulthood an internal plasticity appropriate to the adaptation of motor skills and habits to experience throughout life. The neocortex appears to be a far more complex target for functional reconstruction. Thus, a precise columnar and laminar organization is critical to cortical processing of afferent information, and plans for action are distributed via long-distance projections of cortical outputs that are precisely organized at a topographical level. Although it has proved possible to restore a functional circuitry with repositioning of circumscribed slabs of barrel cortex into the precisely corresponding area in developing brain (Andres and Van der Loos, 1985), more general reorganization following more diffuse lesions has proved far more difficult to reconstruct, without precise long-distance connectivity being restored to and from for example, the thalamus, and without effective functional recovery (Dunnett *et al.*, 1987a; Sofroniew *et al.*, 1990). Although cortical repair has been investigated in far less detail than in the striatum, coherent additive recovery of function by

combination of transplants in multiple systems appears beyond reach, at least at the present state of knowledge.

Rather, our consideration of the experimental basis and principles of striatal repair suggests that we should instead seek to determine whether some rather than other aspects of the symptom profile of HD, or particular stages of disease progression, might be preferentially amenable to striatal repair. Future trials are likely to select earlier stage patients for safety reasons (see Section II D), and this choice is likely to be endorsed by the stage when degeneration is still primarily restricted to the striatum. Also, systematic comparison of individual patient data is likely to indicate certain classes of HD symptoms (e.g., formation and adaptation of motor skills and habits with experience) are more responsive to transplantation than others, suggesting a greater dependence of such functions on the core striatal pathology, as has already proved the case in comparisons in the course of the preparations for a new nigral transplantation program in PD (unpublished data, EU Transeuro consortium). Alternatively, other neurological syndromes might be more suitable for cell transplantation based upon a more selective striatal profile of neurodegeneration – such as certain rare focal ischemias, but in such case the lateralized nature of the disorder with predominant unilateral motor symptoms and little cognitive and psychiatric impairment is unlikely to warrant such speculative surgical intervention.

## IV. Conclusion

In conclusion, striatal grafts can work well to reconstruct the damage and alleviate a range of motor and cognitive deficits in experimental animals with specific striatal lesions. These results provided encouragement for development of a clinical application in HD, and clinical trials in several centers in Europe and North America provide evidence of safety and preliminary data on efficacy. Nevertheless recovery in patients has so far not been seen as reliable or reproducible as has been achieved in many laboratory studies in animals. In part this reflects variability and lack of standardization of clinical protocols, the limited availability of high quality human fetal donor tissue, and the need to develop alternative cell sources for transplantation. However, in part it may also reflect the fact that human HD involves a broader range of pathology than the striatal degeneration that is repaired by striatal transplantation alone. The present chapter illustrates how the critical analysis of the principles and mechanisms for functional recovery may guide patient selection and management, along with optimization of different transplantation methods, which might be expected to maximize the benefits to be achieved by cell transplantation as an important component in an integrated treatment plan designed to meet individual patient needs in this complex neurodegenerative disorder.

## Acknowledgments

Our own studies have been supported by funding from the Medical Research Council, the Welsh Assembly Government, the Cure HD Initiative, and the Lister Institute of Preventative Medicine.

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# CLINICAL PHENOMENOLOGY OF DYSTONIA

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Dystonia is defined as a motor syndrome characterized by sustained muscle contractions, usually producing twisting and repetitive movements or abnormal postures. Dystonia can be present at rest or worsened by action. Dystonia is commonly classified according to age at onset (childhood, adolescent type, and adult type), etiology (idiopathic, and symptomatic), and distribution (focal dystonia, segmental dystonia, generalized dystonia, multifocal dystonia and hemidystonia). The different subtypes of focal and segmental dystonias may have different clinical features. Neuropsychiatric disorders may be present in dystonia.

## I. Historical Review

The first observations of dystonia date back to the beginning of the 20th century. The German physician Oppenheim coined the term dystonia in 1911, describing six patients characterized by clinical features compatible with early-onset generalized dystonia (Oppenheim, 1911). Oppenheim used the term "dystonia" to indicate that "the muscle tone is in certain moments hypotonic, whereas in others it is subject to muscle spasms that are usually induced by voluntary movements."

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00018-3




0074-7742/10 $35.00

He hypothesized that dystonia was an organic disease and enriched the semeiological description with other characteristic signs of this condition: twisted posture of the limbs and the trunk associated with spasms, bizarre gait alterations with bending, flexion, and twisting of the trunk, and rapid and rhythmic jerking movements; he also stated that symptoms tended to evolve into fixed postural deformities. By using two definitions in Latin, he highlighted two main aspects of this disease: *dysbasia lordotica progressiva* was used to refer to the evolutionary nature of the disease with the presence of a pronounced gait alteration (also named *dromedary gait*) and postural abnormalities of the trunk, while *distonia musculorum deformans* was used to refer to the presence of muscle tone alterations leading to long-term trunk and limb postural abnormalities.

However, in subsequent decades, the majority of dystonia cases continued to be considered as patients affected by a conversion reaction (Lesser and Fahn, 1978); only late in the 20th century was an organic framework for dystonia firmly established through the identification of genetic mutations in families with generalized dystonia (Ozelius *et al.*, 1992) and the demonstration that the contralateral basal ganglia are often damaged in patients with acquired hemidystonia (Marsden *et al.*, 1985). At about the same time, it was finally clarified that even some isolated and relatively benign hyperkinetic disorders that occur in adult life (including blepharospasm, oromandibular dystonia, dystonic writer's cramp, torticollis and axial dystonia) are also forms of dystonia, and that there is a continuum from the more severe generalized type to the more benign, strictly focal forms (Marsden, 1976a). Dystonia is now viewed as an organic brain abnormality whose pathogenesis is increasingly being investigated by means of functional brain imaging and electrophysiological and genetic techniques (Berardelli *et al.*, 1988; Colosimo *et al.*, 2005; Perlmutter *et al.*, 1997; Tinazzi, Squintani, and Berardelli, 2009).

## II. Definition and Classification

In 1984, an Ad Hoc Committee of the *Dystonia Medical Research Foundation* developed a definition of dystonia and classification of dystonic movements that is still widely accepted (Fahn, 1987). Dystonia was defined as a motor syndrome characterized by sustained muscle contractions, usually producing twisting and repetitive movements or abnormal postures. It was noted that dystonia is usually precipitated by action, and that almost all dystonic movements share a directional quality that is typically sustained, sometimes even only briefly. The committee classified dystonia according to three main features: age at onset, etiology, and distribution (Fahn, 1987).

The age at onset of dystonia was divided into the childhood type (0 to 12 years), adolescent type (13 to 20 years), and adult type (over 21 years). Age is the most important feature in predicting clinical outcome, with earlier age at onset being

Table I
Main Prognostic Determinants in Dystonia.

- Age at onset
- Etiology
- Body distribution (focal, segmental, hemidystonia, generalized)
- Severity (abnormal postures, jerks, tremor)

associated with both a more extensive spread to other body regions and a more severe clinical picture (Table I).

The etiology of dystonia was divided into two main categories: idiopathic, when no exogenous cause or brain lesions are identified, and symptomatic, when an exogenous cause that may be ascribed to a variety of origins (e.g. structural lesions, drugs/toxins, and metabolic disorders) is identified (Table II). Recent advances in genetics have, however, demonstrated that many forms of idiopathic dystonia are genetic in nature. Idiopathic dystonia should therefore be renamed "primary" and symptomatic dystonia "secondary." A subcategory of complex

Table II
Etiology of Dystonia.

Primary (idiopathic)
  Sporadic
  Familial
Secondary (symptomatic)
  Dystonia-plus
    Dopa-responsive dystonia (Segawa's syndrome)
    Rapid-onset dystonia parkinsonism
    Myoclonus-dystonia
  Heredodegenerative dystonia
    Autosomal dominant (e.g. HD, SCA3, DRPLA)
    Autosomal recessive (e.g. Wilson disease, GM1, and GM2 gangliosidosis, homocystinuria)
    X-linked (e.g. X-linked dystonia parkinsonism/Lubag)
  Acquired causes
    Drug-induced (e.g. tardive dystonia)
    Basal ganglia lesions (particularly the putamen) due to stroke, tumor, vascular malformations, demyelination, etc.
    Intracranial or peripheral trauma
  Unknown etiology
    Parkinson's disease
    Corticobasal degeneration
    Multiple system atrophy
    Progressive supranuclear palsy

secondary dystonic syndromes, the so-called dystonia-plus, have also been identified (Table III). These forms are characterized by other neurological features in addition to dystonia (e.g. parkinsonism and spasticity in DOPA-responsive dystonia, or myoclonus in myoclonic dystonia linked to mutations in the epsilon-sarcoglycan gene), though without any apparent neurodegeneration, and need to be differentiated from other dystonic syndromes (Kurlan *et al.*, 1988; Quinn, Rothwell, and Thompson, 1988; Segawa, 2010).

The classification of dystonia by distribution was based on the following categories: focal dystonia, segmental dystonia, generalized dystonia, multifocal dystonia, and hemidystonia (Table IV). The term focal dystonia refers to the

Table III
Combination of Physical Signs Observed in Different Dystonia Syndromes.

| Dystonia Syndromes | Physical Signs Observed |
|---|---|
| Primary (or idiopathic) dystonias | Dystonia |
| Dystonia plus syndromes | Dystonia, parkinsonism, myoclonus |
| Paroxysmal dystonias | Dystonia, chorea, myoclonus |
| Heredodegenerative dystonias | Dystonia, parkinsonism, chorea, myoclonus, spasticity, cerebellar features, dysautonomia, cognitive impairment, epilepsy |
| Symptomatic dystonias | Same as for heredodegenerative dystonias plus focal neurological signs, if present |

From: Albanese and Lalli (2009).

Table IV
Clinical Classification of Dystonia by Affected Body Part.

| Type of Dystonia | Affected Part |
|---|---|
| Focal | [A single body part is affected]. Examples:<br>Eyelids (blepharospasm)<br>Mouth (oromandibular dystonia, *embouchure* dystonia)<br>Larynx (spasmodic dysphonia)<br>Neck (cervical dystonia)<br>Hand and arm (including occupational cramps) |
| Segmental | Cranial (two or more parts of cranial and neck musculature)<br>Axial (neck and trunk)<br>Brachial (one arm and axial; both arms ± neck ± trunk)<br>Crural (one leg and trunk; both legs ± trunk) |
| Generalized | A combination of segmental crural and any other segment |
| Multifocal | Two or more noncontiguous parts |
| Hemidystonia | Arm and leg on one side |

involvement of a single body part. Blepharospasm, oromandibular dystonia, spasmodic dysphonia, spasmodic torticollis, and writer's cramp are all forms of focal dystonias. Segmental dystonia is characterized by the involvement of two or more contiguous regions of the body. The segmental forms are subdivided into regional categories: cranial, axial, brachial, and crural (Fahn, 1987; Marsden, 1976b). Cranial dystonia is characterized by the involvement of any combination of musculature in the head and neck region, though the neck and mandible do not belong to the cranium anatomically speaking. It may thus be more appropriate to classify blepharospasm, masticatory dystonia, and cervical dystonia (CD) as "craniocervical" dystonia as opposed to "cranial" dystonia (Colosimo *et al.*, 2010). Generalized dystonia, which is the most severe form of dystonia, consists of segmental crural dystonia and dystonia in at least one additional body part (Marsden and Harrison, 1974).

Childhood onset dystonia tends, unlike the adult onset form of this disorder, to progress into generalized dystonia. In addition, familial aggregation is more common in early-onset forms, whereas late-onset primary dystonia is often sporadic and tends, at least initially, to remain focal or segmental in distribution (Defazio, Berardelli, and Hallett, 2007). The classical example of childhood-onset generalized dystonia is the disease due to the DYT1 gene mutation, which is also referred to as Oppenheim's dystonia. DYT1 dystonia is an autosomal-dominant disorder that is caused, in the vast majority of cases, by a GAG deletion in the *TOR1A* gene (whose final product is *Torsin A*) and is typically characterized by early onset in a limb, generalization, and a tendency to spare the cranial-cervical muscles (Ozelius *et al.*, 1992). Patients with this disease have symptom onset before the 30 years of age and normal cognitive function. The functional prognosis in children with this form is usually severe (Fig. 1), with a considerable proportion of

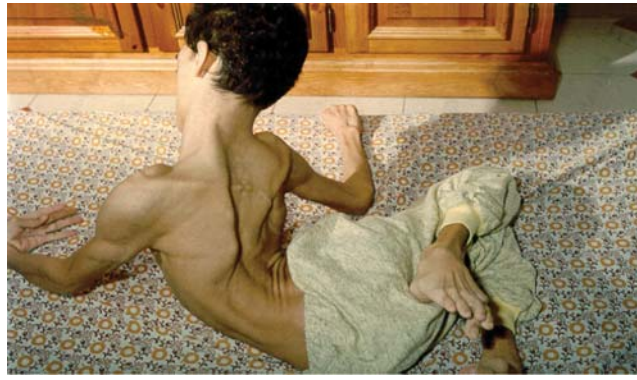

FIG. 1. Image of a patient affected by severe DYT-1 generalized dystonia. Courtesy of Dr. AR Bentivoglio, Rome.

patients becoming wheelchair-bound or requiring walking aids before they reach adult age (Anca *et al.*, 2003). Fortunately, therapies that have been developed over the last decade and are based on a combination of drugs, botulinum toxin, and functional neurosurgery now allow patients with Oppenheim's dystonia to be more independent and to have a better quality of life than was previously possible. A few exceptions to this typical presentation have been reported, describing mutation carriers from DYT1 families who present with focal or segmental dystonia of adult onset (Edwards *et al.*, 2003). Family studies have also assessed that the penetrance of DYT1 dystonia is only around 30%. Conversely, patients with early-onset primary dystonia not caused by the DYT1 gene tend to have later age at onset, less commonly limb onset, and overall a slower progression than DYT1 primary dystonia cases (Albanese *et al.*, 2011).

Multifocal dystonia is characterized by the involvement of two or more non-contiguous parts. Hemidystonia is a syndrome involving only half of the body, and is usually due to a structural lesion with various etiologies in the contralateral basal ganglia (Factor, Troche-Panetto, and Weaver, 2003; Marsden *et al.*, 1985; Pettigrew and Jankovic, 1985): hemiparesis and pyramidal signs are not uncommon, though the clinical course of this unusual disorder is nonprogressive.

The risk of mistaking some cases of adult-onset dystonia with arm tremor, including a rest component and reduced arm swing on the affected side, for Parkinson's disease (PD) may be high. Clinicians should consequently be aware that primary adult-onset dystonia may present with an asymmetric resting arm tremor, impaired arm swing and sometimes facial hypomimia or jaw tremor, though without evidence of true bradykinesia (i.e., with progressive fatiguing). Given the serious consequences of misdiagnosing such cases as PD patients, functional imaging with a dopamine transporter tracer should always be performed in cases in which the diagnosis is uncertain (Schneider *et al.*, 2007). Since the result of this examination in dystonia patients is negative, dystonic tremor in cases suspected of being affected by PD should always be considered as a possible cause of the so-called *Scans Without Evidence of Dopaminergic Deficit* (*SWEDDs)*.

Dystonia is also one of the most common presentations of psychogenic movement disorders (PMD). The term "psychogenic" is used to describe disorders that cannot be attributed to a known underlying organic cause. The identification of such cases still poses a major challenge in neurological practice. Although PMD is traditionally diagnosed by excluding other diseases, recent clinical and physiological advances have led to the establishment of some diagnostic criteria for this disease (Gupta and Lang, 2009). The diagnosis of PMD is definite when symptoms resolve following suggestion therapy, psychotherapy, physiotherapy, or the administration of a placebo, or when the patient's symptoms disappear upon being left alone and unobserved (Fahn and Williams, 1988). Unfortunately, the prognosis of psychogenic dystonia remains disappointing, with the majority of patients suffering significant long-term disability.

Lastly, dystonia may present in the form of primary paroxysmal disorders. These rare disorders are classified as idiopathic (often familial) or symptomatic and they may be ascribed to a variety of causes (Albanese *et al.*, 2011). Three main forms with different triggering factors are known: in paroxysmal kinesigenic dystonia attacks are induced by sudden movement, in paroxysmal exercise-induced dystonia by intense exercise, such as walking or swimming, and in the nonkinesigenic form by alcohol, coffee, or tea drinking.

To sum up, dystonia is a disease whose severity at onset, natural history and impact on the patient's quality of life may vary; consequently, several factors need to be taken into consideration when a prognosis of functional impairment is made in a newly diagnosed patient.

## III. Clinical Features in Different Subtypes of Focal and Segmental Dystonia

### A. Blepharospasm

Blepharospasm is a common adult-onset focal dystonia, characterized by involuntary contractions of the periocular muscles resulting in forceful eye closure, which impairs normal opening and closing of the eyes (Fabbrini *et al.*, 2009; Grandas *et al.*, 1988; Jankovic and Orman, 1984; Marsden, 1976a). The muscle invariably involved in blepharospasm is the orbicularis oculi (Berardelli *et al.*, 1985). The orbicularis oculi, a sphincter muscle around the eye, is composed of orbital, preseptal, and pretarsal parts. These parts play different roles in the mechanism of eye closure. Blepharospasm may also involve other muscles, such as levator palpebrae superioris, corrugator, procerus, and frontalis.

Blepharospasm is primary in the majority of cases, and secondary in the few cases in which it is due to structural brain lesions or is drug induced (Jankovic, 2006; Tolosa, 1993). The severity of blepharospasm may vary from repeated frequent blinking, which causes only minor discomfort, to persistent forceful closure of the eyelids, which leads to functional blindness (Fig. 2). The characteristic features of blepharospasm include sensory tricks that patients use to relieve their symptoms (*geste antagoniste*), such as rubbing of the eyelids, and frequent ocular symptoms (related to diseases of the anterior ocular segment, for example, blepharitis and keratoconjunctivitis) that occur either before or at the onset of the spasm (Calace *et al.*, 2003; Marsden, 1976b). Blepharospasm may be associated with levator palpebrae superioris muscle inhibition (eyelid opening apraxia) or involuntary movements in the lower face or jaw muscles (Meige syndrome). Blepharospasm and eyelid opening apraxia may be associated with specific neurodegenerative conditions, such as progressive

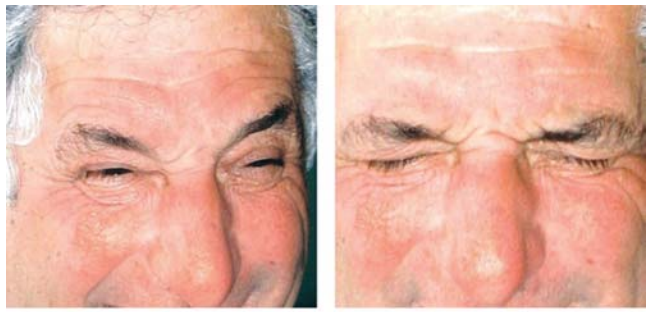


FIG. 2. Images of a patient affected by blepharospasm. (a) and (b) show different phases of an involuntary bilateral orbicularis oculi muscle contraction. From: Fabbrini *et al.* (2009).

supranuclear palsy and corticobasal degeneration (Colosimo *et al.*, 2011; Defazio, Berardelli, and Hallett, 2007).

Differences in the risk of spread between the various forms of focal dystonia over time and the influence of age at dystonia onset on the risk of spread are not well established. In an Italian multicenter survey, age at dystonia onset, age at initial spread and the risk of initial spread were found to be significantly higher in blepharospasm patients than in patients with onset in the neck or upper extremities, whereas the time elapsing from onset to initial spread was significantly lower (Abbruzzese *et al.*, 2008). The increased risk of spread in the blepharospasm group was most evident in the first 5 years following onset, after which it declined and became similar to that of patients with CD or limb dystonia.

## B. MEIGE'S SYNDROME

In 1910, the French neurologist Henri Meige described ten patients with involuntary closure of the eyelids (Meige, 1910). Blepharospasm in one of these patients was associated with involuntary contractions of the jaw muscles. This eponym became popular a few decades later and the possibility that the pathophysiology of blepharospasm and oromandibular dystonia is shared was hypothesized (Paulson, 1972). In 1976, David Marsden first drew attention to "De Gaper" ("The Yawning man", Musées Royaux des Beaux-Arts, Bruxelles), a work of art by the celebrated painter Pieter Brueghel the Elder, in an article on blepharospasm and oromandibular dystonia (Marsden, 1976a), despite the fact that Brueghel's vivid painting of a yawning subject has nothing to do with dystonia. It has been suggested that the essential sign of the Brueghel syndrome

is "a widely and dystonically opened jaw." However, although jaw-opening dystonia has been associated with blepharospasm, it may otherwise occur in the setting of segmental, multifocal, or generalized dystonia (Fabbrini *et al.*, 2009). Indeed, the application of the Brueghel syndrome to all these cases should be considered misleading and confusing. Consequently, while the eponymic term "Meige's syndrome" is still used, the term Brueghel syndrome has now become obsolete (LeDoux, 2009).

### C. Oromandibular Dystonia

Dystonic spasms may affect the lower facial and jaw muscles (oromandibular dystonia, OMD) and are often associated with blepharospasm (Marsden, 1976b). OMD most often manifests itself as closing dystonia, though it may also be characterized by opening jaw dystonia (also called Brueghel syndrome, see the previous paragraph) or deviation and retraction of the jaw, or a combination of these movements (Fabbrini *et al.*, 2009). OMD is, on rare occasions, unilateral, causing deviation of the jaw to one side. The spasms frequently also involve muscles of the mouth and tongue. OMD may arise spontaneously or during eating, chewing or speaking, and render communication and nutrition difficult. Examination may disclose a variety of antagonist maneuvers or sensory tricks, including touching the lips or chin, chewing gum, or biting on a toothpick. OMD is often a socially embarrassing and disfiguring condition. Embouchure dystonia is a task-specific cranial dystonia typical of musicians. It involves the muscles used to initiate and control the amplitude and force of airflow into the mouthpiece of a woodwind or brass instrument. The most common phenotype is involuntary, task-specific tremor of the lips, while less common patterns include involuntary lip movements and jaw closure (Fabbrini *et al.*, 2009). This rare disorder may be severe enough to hinder the career of some professional musicians.

### D. Lingual Dystonia

Primary focal lingual dystonia is a rare disorder that manifests itself in the form of action dystonia during speech or through paroxysmal episodic lingual spasms (Fabbrini *et al.*, 2009). It may be so severe as to interfere with speech, swallowing and breathing. Tardive lingual dystonia, secondary to the use of dopamine receptor blocking agents, may manifest itself in a relatively isolated form (Schneider *et al.*, 2006). Severe tongue protrusion, particularly during eating, is characteristic of neuroacanthocytosis (Bader *et al.*, 2010) but may also be seen in other rare and fatal neurodegenerative diseases, such as pantothenate kinase-associated neurodegeneration and Lesch–Nyhan syndrome (Schneider *et al.*, 2006).

### E. Laryngeal Dystonia

Laryngeal dystonia (also commonly referred to as spasmodic dysphonia) is a neurological voice disorder characterized by involuntary adductor (towards the midline) or abductor (away from the midline) vocal fold spasms during phonation, which result in phonatory breaks and consequent articulatory speech disorder (Marsden, 1976a). The most common form of laryngeal dystonia is adductor spasmodic dysphonia. Some patients also have a mixed form of this disorder. Laryngeal dystonia often begins late in life and may be either mild or severely disabling; the latter form leads to enduring impairment in verbal communication and reduced quality of life (Murry *et al.*, 1994).

### F. Cervical Dystonia

CD (also called spasmodic torticollis) is the most common form of focal dystonia, its prevalence being approximately 6 per 100,000 (Nutt *et al.*, 1988). The mean age at onset is in the fifth decade. Cervical dystonia is a lifelong chronic disorder with a varying clinical presentation and progression (Lowenstein and Aminoff, 1988), though longitudinal studies have unequivocally shown that spontaneous remission rarely occurs, and tends to be transient when it does (Jayne, Lees and Stern, 1984). Cervical dystonia is characterized by involuntary postures of the head due to involuntary spasms, jerks or tremors (or all three combined) and is frequently associated with neck pain (Hughes, Lees and Marsden, 1991). A common clinical classification of CD is based on the type of movement and position of the head in affected patients, the most common type being rotational torticollis (>50%). Other relatively common patterns include laterocollis (i.e., lateral flexion) and retrocollis (i.e., posterior flexion), whereas anterocollis (i.e., anterior flexion) and complex forms of CD (in which no predominant component can be identified) are less common (Colosimo *et al.*, 2010). Most patients, however, display a combination of various abnormal patterns, even when a main component is identified. Interestingly, there is no significant preponderance of right or left deviation. Moreover, abnormal posture is present most of the time in CD patients, though it may change markedly during the illness and may even rapidly reverse direction (Chan *et al.*, 1991). CD commonly involves several neck muscles, including the sternocleidomastoid, splenius, trapezius, levator scapulae, scalenii, and semispinalis muscles. Various sensory tricks, such as touching the face contralaterally or ipsilaterally to the direction of head rotation (Fig. 3), may reduce the involuntary neck movements, albeit temporarily. As in other forms of focal dystonia, stress exacerbates while relaxation improves the symptoms of CD. With the introduction of botulinum toxin therapy, many of the severe long-term complications of CD, such as contractures, radiculopathies, and

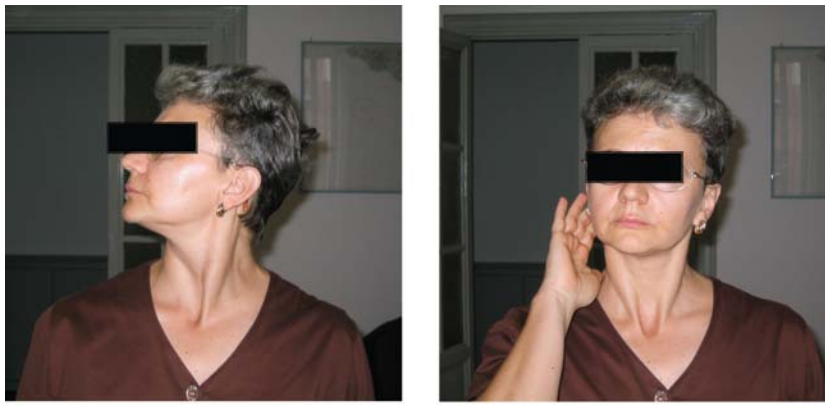


FIG. 3. Images of a patient affected by cervical dystonia (rotational type). Note the hypertrophy of the sternocleidomastoid muscle contralateral to the head rotation (a), and a typical sensory trick (b).

compressive cervical myelopathy, have become less common (Jankovic and Orman, 1987; Ward *et al.*, 2006). Cervical dystonia is usually primary in nature (in which case all investigations, including brain neuroimaging, are normal), though rare cases secondary to basal ganglia brain lesions have may occur.

## G. Limb Dystonia

The legs are frequently involved in early-onset generalized dystonia, whereas the limbs in general are rarely affected in adults with focal or segmental dystonia. Adult forms usually first manifest themselves as action dystonia, being only occasional the secondary cases of limb dystonia that start as dystonia at rest. During a volitional movement of the affected limb is also noticeable another characteristic feature of dystonia, the "overflow"' of contractions to adjacent or remote muscles.

Task-specific, focal limb dystonia is often seen in occupational cramps (Roze *et al.*, 2009). This type of focal limb dystonia occurs in associations with specific repetitive actions such as writing, typing, feeding, in some sports (e.g., golf) and when musical instruments are being played, particularly by professional musicians who are subjected to particularly long training sessions. Primary lower limb dystonia is a rare and often misdiagnosed condition: results from a recent multicenter study, based on a series of consecutive out-patients with adult-onset

primary dystonia attending nine Italian teaching hospitals, showed that out of the 579 patients assessed, only 11 (1.9%) had lower limb dystonia (Martino *et al.*, 2010).

### H. Axial Dystonia

Axial or trunk dystonia is a rare dystonic disorder that can result in scoliosis, lordosis, kyphosis, tortipelvis, and opisthotonic posturing (or a combination of these abnormalities). This is a severe disorder that may start as action dystonia (e.g. seen only in walking or running), and later progress to a fixed deformity that persists even when the patient is at rest (Bhatia, Quinn and Marsden, 1997). These patients generally share a number of clinical features with other types of adult onset primary dystonias. They have no family history of dystonia and tend to remain focal, although some contiguous spread may occur. If spread does occur, involvement of the head, neck, and arms is mild in comparison with the severe trunk dystonia. Since treatment response to various drugs and to botulinum toxin is generally poor, the functional prognosis is consequently also poor, and severe depression may affect a significant number of these patients, owing mainly to the negative personal image resulting from such a disfiguring disease.

## IV. Neuropsychiatric Features of Dystonia

It has now become clear that dystonia is not merely a motor system disorder. Indeed, the association between depression and obsessive-compulsive behavior and various forms of dystonia has been the subject of numerous studies conducted by research groups throughout the world. In a study performed by one centre in Austria, 14% of the patients with CD were found to have moderate to severe depression (according the Beck depression index), which improved significantly after botulinum toxin treatment (Müller *et al.*, 2002). The occurrence of frequent psychiatric disorders with various forms of focal dystonia (including blepharospasm and CD) was confirmed in a recent study on 89 patients conducted by our group (Fabbrini *et al.*, 2010). When patients were assessed by means of a structured clinical interview for DSM-IV (SCID-I) and other standardized psychiatric scales, we found that depressive disorders were more frequent in the blepharospasm and CD groups than in healthy controls, whereas the frequency of anxiety and obsessive-compulsive or adjustment disorders was comparable to that of healthy subjects. Findings from a series of noteworthy studies by Jahanshahi and Marsden (1988 and 1990a) also support the concept that depression in patients affected by dystonia is largely related to a negative body image, due to abnormal postures of the head and other body parts (Jahanshahi and Marsden, 1990b). Indeed, perceived stigma and consequent social avoidance behaviur are well-

known psychosocial features of CD that very likely contribute to the development of depression (Papathanasiou *et al.*, 2001).

## V. Conclusions

Dystonia is a term used to refer to a common movement disorder in humans and to identify a heterogeneous group of primary (idiopathic) and secondary (symptomatic) disorders of this kind. Diagnosing dystonia may be difficult owing to the variability of its clinical presentation, unclear recognition of its clinical signs, the wide range of etiological factors and the coexistence of other movement disorders. The main difficulties encountered in the diagnostic assessment of dystonia are often due to the confusion that arises between this disorder and other organic movement disorders or psychogenic syndromes. The movement disorders that may most commonly be mistaken for dystonia are essential tremor, PD, myoclonus, chorea, tics, and conversion reactions. Specific diagnostic algorithms, together with genetic and laboratory tests, are essential to recognize the clinical signs of dystonia, as recommended in recent international consensus guidelines (Albanese *et al.*, 2011). Because of the lack of specific diagnostic tests, careful clinical observation is always recommended, whereas appropriate investigations are required if the initial presentation or the natural history of the disease suggests heredodegenerative or secondary (symptomatic) dystonia.

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# GENETICS AND PHARMACOLOGICAL TREATMENT OF DYSTONIA

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Dystonia consists of involuntary repetitive twisting (torsion) or directional movements, sometimes leading to sustained postures. The movements are stereotyped and characterized by co-contraction of agonist and antagonist muscles. There is a broad clinical spectrum of dystonia which derives in part from the differential distribution of involvement. Dystonia may be localized, affecting a single body region, or generalized, affecting multiple extremities along with the trunk. Intermediate dystonic involvement can be described as segmental, designating two affected contiguous body regions, or multifocal, designating two or more noncontiguous affected body regions. Hemidystonia refers to dystonia affecting only one side of the body.

Dystonia can also be categorized by age of onset and etiology. Early onset dystonia, occurring in childhood or adolescence (in some studies younger than 26 years old), is associated with more progressive disease [Greene *et al.* (1995). *Mov. Disord.* **10**, 143]. In this age group, dystonia usually first appears in a limb and then spreads to involve other limbs and axial muscles; some early-onset patients may have involvement of laryngeal and other cranial muscles. Adult or late-onset dystonia typically begins in the neck, arm, or cranial muscles. Compared to early-onset dystonia, the area of involvement is more likely to remain focal or segmental. Dystonia can be considered either primary or nonprimary. Primary torsion dystonia (PTD), historically called dystonia musculorum deformans and Oppenheim's dystonia, describes dystonia in isolation, excepting tremor, without brain degeneration and without an identified acquired cause. Nonprimary or secondary dystonia encompasses a heterogeneous group of syndromes and etiologies including inherited (with or without brain degeneration), acquired, and complex neurological disorders.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00019-5




0074-7742/10 $35.00

Monogenic forms of dystonia are labeled DYT and enumerated in the order in which they were discovered. The current 20 DYT loci comprise a heterogeneous group of disorders. (Table I) They can be divided into PTDs, dystonia-plus syndromes without brain degeneration, dystonia-parkinsonism with brain degeneration (i.e. DYT3), and paroxysmal dyskinesias. There are many neurodegenerative genetic disorders that share dystonia as a common feature of disease (Table II). This chapter will review the genetics of PTD, dystonia-plus syndromes without brain degeneration, and X-linked dystonia-parkinsonism. Other genetic dystonia-parkinsonism syndromes and the paroxysmal dyskinesias will not be discussed.

## I. Primary Torsion Dystonia

In early-onset PTD, symptoms typically first appear in a leg or arm, spread to other limbs and the trunk, and culminate in generalized or multifocal muscle involvement. A large proportion of early-onset PTD is attributable to a heterozygous mutation in *TOR1A* (DYT1). DYT2, another familial form of PTD with a phenotype similar to DYT1, has been identified. DYT2, which has been described in a limited number of families, is inherited in an autosomal recessive fashion and its locus has not been mapped.

In a less common early-onset PTD phenotype, dystonia tends to begin in the arm, neck, or cranial muscles, before becoming generalized or multifocal. Unlike DYT1 there is often significant speech impairment due to cranial muscle involvement. The combination of early onset and a tendency for the cranial and cervical muscles to be affected, like late-onset PTD, led to the designation of a mixed or intermediate phenotype. Three gene loci, DYT6, DYT13, and DYT17, have been identified in families with a predominant intermediate phenotype. Although late-onset focal cervical and cranial dystonia are the most common forms of PTD, about 9–10 times more prevalent than generalized PTD, only one locus for this phenotype has been identified, DYT7.

### 1. *DYT1 Dystonia*

The most common known genetic cause of PTD, DYT1 dystonia, is inherited in an autosomal dominant pattern with approximately 30% penetrance and variable expression. Common clinical characteristics have been described across ethnic groups (Bressman *et al.*, 2000). The majority of manifesting mutation carriers presents between the ages of 3 and 26 with arm or leg symptoms. In some cases, the dystonia may be jerky or tremulous, mimicking tremor. Dystonia spreads over 5 to 10 years, with about 70% progressing to generalized or multi-focal dystonia, while the rest continue to have focal (20%) or segmental dystonia

Table I
Molecular Classification of Dystonia.

| Designation | Dystonia Type | Inheritance | Gene Locus | Gene/Product | OMIM Number |
|---|---|---|---|---|---|
| DYT1 | Early-onset generalized primary torsion dystonia (PTD) | AD | 9q | GAG deletion in DYT1 coding for torsinA | 128100 |
| DYT2 | Autosomal recessive PTD | AR | Unknown | Unknown | 224500 |
| DYT3 | X-linked dystonia parkinsonism; "lubag" | XR | Xq | *TAF1/DYT3* | 314250 |
| DYT4 | "Non-*DYT*1" PTD with whispering dysphonia | AD | Unknown | Unknown | 128101 |
| DYT5/ DYT14 | Dopa-responsive dystonia; | AD | 14q | *GCH1*/GTP-cyclohydrolase | 128230 |
| | Segawa syndrome | AR | 11p | Tyrosine hydroxylase | |
| DYT6 | Adolescent-onset "mixed" type PTD | AD | 8p | *THAP1* | 602629 |
| DYT7 | Adult-onset focal PTD | AD | 18p | Unknown | 602124 |
| DYT8 | Paroxysmal nonkinesigenic dyskinesia (PNKD) | AD | 2q | Myofibrillo-genesis regulator 1 | 118800 |
| DYT9 (DYT18) | Paroxysmal choreoathetosis with episodic ataxia and spasticity | AD | 1p | *GLUT1/SLC2A1* | 601042 |
| DYT10 | Paroxysmal kinesigenic dyskinsia/choreoathetosis (PKD) | AD | 16p-q | Unknown | 128200 |
| DYT11 | Myoclonus-dystonia | AD | 7q | *SGCE*/epsilon-sarcoglycan | 159900 |
| DYT12 | Rapid-onset dystonia-parkinsonism | AD | 19q | *ATP1A3*/Na/K ATPase alpha 3 | 128235 |
| DYT13 | Early-onset Multifocal/ segmental PTD | AD | 1p | Unknown | 607671 |
| DYT15 | Myoclonus-dystonia | AD | 18p | Unknown | 607488 |
| DYT16 | Dystonia-Parkinsonism | AR | 2q31 | *PRKRA* | 612067 |
| DYT17 | Adolescent onset mixed PTD | AR | 20p11.22-q13.12 | Unknown | 612406 |
| DYT18 | Paroxysmal exercise - induced dystonia (PED) | AD | 1p35-31.3 | *GLUT1/SLC2A1* | 612126 |
| DYT19 | Episodic kinesigenic dyskinesia 2 | AD | 16q | Unknown | 611031 |
| DYT20 | Paroxysmal nonkinesigenic dyskinesia 2 | AD | 2q | Unknown | 611147 |

(10%). When the final distribution of dystonia is analyzed, one or more limbs are almost always affected with over 95% having an affected arm. The trunk and neck may also be affected (25–35%) and may cause the greatest disability. The cranial muscles are less likely to be involved (<20%), and in one study of early-onset PTD, cranial involvement was the best clinical predictor of non-DYT1 status (Fasano *et al.*, 2006).

Rarely, DYT1 family members have been identified with late-onset dystonia. These individuals generally have focal disease, are identified in the course of family studies, and often do not seek medical attention. Although the arm is the body region most commonly affected in those with focal disease, the neck or cranial muscles have been reported as isolated affected sites (Bressman *et al.*, 2000; Leube *et al.*, 1999; Tuffery-Giraud *et al.*, 2001). Isolated cervical or cranial dystonia from DYT1 mutations, however, is quite rare. Indeed, one study of patients with early-onset cervical dystonia failed to find any DYT1 mutations (Koukouni *et al.*, 2007), and another study found that DYT1 mutations are very rarely associated with adult focal dystonia (Jamora *et al.*, 2006).

The causative gene, TOR1A, maps to chromosome 9q34, and encodes the protein torsinA, a 332 amino acid protein (Ozelius *et al.*, 1997). There is one common pathogenic TOR1A mutation, a GAG deletion in exon 5. The deletion results in loss of a glutamic acid residue at amino acid position 302 or 303. Other coding variations in DYT1 have been found, but their pathogenicity is unclear: an 18-bp deletion (966_983del) in a single atypical family that also harbored a mutation in epsilon sarcoglycan (Leung *et al.*, 2001); a 4-base pair deletion (934_937delAGAG) which causes a frameshift and truncation starting at residue 312 identified in a single control blood donor not examined neurologically (Kabakci *et al.*, 2004); and G>A transition at position 863 (G863A) resulting in substitution of arginine for glutamine in a single patient with severe fixed dystonia, facial palsy, and long tract signs, with symptoms beginning in infancy (Zirn *et al.*, 2008). There is stronger evidence for two other variants that produce coding changes. One is a SNP that results in the substitution of an aspartate with a histidine (D216H) in exon 4; this change appears to modify penetrance of DYT1 (see below). The other is a missense change is exon 3 that results in a change of isoleucine to phenylalanine (c.613T>A, p.F205I). This change was identified in a man with bipolar disease who presented with orobulbar dystonia in his forties. His clinical picture was complex as he was taking lithium and had a history of neuroleptic exposure; further, on examination, there was cogwheeling and an action tremor without rest tremor. The effect of the mutation was tested in a cellular assay and produced intracellular inclusions, similar to those seen with GAG deletions (Calakos *et al.*, 2010).

Owing to a founder effect and genetic drift, the DYT1 GAG deletion accounts for about 80% of early-onset generalized PTD in those with Ashkenazi ancestry compared to 16–53% in non-Jewish populations. The gene frequency of the

DYT1 GAG deletion among Ashkenazi Jews was estimated in one study to be 1/2000 –1/6000 (giving a carrier frequency of 1/1000–1/3000) (Risch *et al.*, 1995); based on a penetrance of 30%, this translates into a disease frequency of 1/3,000–1/9,000. A recent study from southeastern France, using direct genotyping of 12,000 newborn dried blood samples, identified one disease allele (Frederic *et al.*, 2007). This carrier incidence of 1/12,000 is consistent with the approximately fivefold increased frequency of early-onset PTD in Ashkenazim compared to non-Jews advanced in older studies, prior to gene identification (Zeman and Dyken, 1967). These studies also imply that a significant proportion of early-onset PTD cases, especially among non-Ashkenazim, are not due to DYT1 mutations.

TorsinA is a member of the AAA+ superfamily (ATPases associated with a variety of cellular activities). These proteins are characterized by $Mg^{++}$-dependent ATPase activity and typically form six-membered, homomeric ring structures. Many serve as chaperones that mediate conformational changes in target proteins. They are associated with a variety of functions including protein folding and degradation, cytoskeletal dynamics, vesicle recycling, and membrane trafficking (Vale, 2000). TorsinA is widely distributed in the brain but restricted to neurons. Although most pathological studies of DYT1 brains have not detected specific morphological changes, one study of 4 DYT1 brains found perinuclear inclusion bodies in the midbrain reticular formation and periaqueductal grey matter, including cholinergic neurons of the pedunculopontine nucleus (McNaught *et al.*, 2004).

Most of the wild-type torsinA protein appears to be located in the lumen of the endoplasmic reticulum (ER) and shuttles between the ER and the nuclear envelope (NE), while the mutant protein is more often associated with the NE (Goodchild and Dauer, 2004; Hewett *et al.*, 2006; Naismith *et al.*, 2009). In cellular models of mutant torsinA, the NE displays abnormal morphology with thickening and whorled membrane inclusions that appear to "spin off" the ER/NE. It has also been shown that mutant torsinA interferes with the linkage between the ER/NE membranes and the cytoskeleton. This may ultimately result in dysfunctional neurite growth (Hewett *et al.*, 2006, 2007; Kamm *et al.*, 2004; Nery *et al.*, 2008). Other cellular effects of the mutation include impaired interactions with major binding partners (Naismith *et al.*, 2009).

Mutant torsinA's impaired protein interactions and aberrant localization may alter synaptic vesicle and dopamine transporter trafficking, dopamine uptake, and dopamine release (Granata *et al.*, 2008; Misbahuddin *et al.*, 2005; Torres *et al.*, 2004). This is supported by DYT1 mouse models (see below) that point to abnormalities in dopamine transport and dopamine signaling (Hewett *et al.*, 2010). TorsinA mutations may also affect dopamine synthesis with neuronal models showing sequestration of tyrosine hydroxylase in inclusions and altered enzyme activity (O'Farrell *et al.*, 2009). How these abnormalities relate ultimately to human disease expression is unclear, as levodopa and dopamine agonists do not

have a robust therapeutic effect in DYT1 patients; however, a functional defect in dopamine signaling is consistent with the efficacy of GPi DBS and anticholinergic medications.

In addition to cellular models there are several mouse models of DYT1 dystonia. These include transgenic models overexpressing mutant human torsinA and knock-in mouse models with modified endogenous expression. In a transgenic model, about 40% of the mice showed motor hyperactivity, with circling and abnormal movements (Shashidharan *et al.*, 2005). The mouse brains demonstrated abnormal levels of dopamine metabolites and brainstem aggregates similar to those reported in DYT1 human brains. Another transgenic model expressing human mutant torsinA was associated with impaired motor sequence learning on the rotorod but no overt movement disorder (Sharma *et al.*, 2005). Knock-in (KI) mice bearing the three base pair deletion in the heterozygous state manifest hyperactivity in the open field, difficulty in beam walking, but no overt dystonic posturing (Dang *et al.*, 2005). The knock-down (KD) mouse model, in which a reduced level of torsinA protein is expressed, displays a phenotype very similar to the heterozygous KI mice. Both models show decreased dopamine metabolite levels (Dang, Yokoi *et al.*, 2006).

In contrast, mice with a homozygous KI deletion and torsinA null mice die at birth with apparently normal morphology. Their postmigratory neurons, however, show NE abnormalities (Goodchild *et al.*, 2005). The fact that both the torsinA null and homozygous KI animals display the same lethal phenotype and that the KD and heterozygous KI display a similar phenotype suggests that DYT1 dystonia results from a loss of function of the torsinA protein. The loss of function could result from a dominant negative effect whereby the mutant protein interferes with or otherwise diminishes the wild-type protein. This is supported by cellular studies that demonstrate inhibition of wild-type protein by mutant torsinA (Torres *et al.*, 2004; Hewett *et al.*, 2006). It has also been shown that mutant torsinA destabilizes wild-type protein leading to premature degradation through the macroautophagy pathway and by the proteasome (Giles *et al.*, 2008; Naismith *et al.*, 2009).

The transgenic mouse model has also yielded insights into possible pathophysiologic mechanisms of disease. A study examining dopamine D2 receptor (D2R) transmission demonstrated a link between the *TOR1A* mutation and D2R dysfunction and found that pharmacological blockade of adenosine A2A receptors restored the abnormal plasticity seen in DYT1 mice. This suggests that antagonism of A2A receptors can counteract abnormal D2R-mediated transmission in mutant mice (Napolitano *et al.*, 2010). Investigation of cholinergic interneurons in the same mutant mouse model showed that activation of D2R was associated with a paradoxical excitatory response which likely resulted in increased acetylcholine release (Pisani *et al.*, 2006). This finding may correspond to the benefit observed clinically from anticholinergic medications.

As stated above, DYT1 dystonia has only 30% penetrance for clinically evident dystonia. With the identification of the disease gene, it is now clear that unaffected *TOR1A* mutant gene carriers may have disease expression in the absence of overt motor signs. Comparing nonmanifesting family members to their noncarrier family members as well as those manifesting dystonia, it was found that both manifesting and nonmanifesting gene carriers had the same increased risk for early onset recurrent major depression when compared to their noncarrier related family members but no differences in OCD frequency (Heiman *et al.*, 2004; Heiman *et al.*, 2007).

Subclinical expressions of DYT1, or endophenotypes, have been investigated using various imaging and neurophysiological approaches with the goal of illuminating pathophysiologic mechanisms that take a gene carrier from "nonmanifesting" to manifesting. Nonmanifesting gene carriers show deficits in specific motor sequence learning paradigms (Ghilardi *et al.*, 2003), and imaging studies reveal microstructural changes involving cerebellothalamocortical fiber tracts (Argyelan *et al.*, 2009; Carbon *et al.*, 2004, 2009), decreased striatal D2 receptor availability (Asanuma *et al.*, 2005; Carbon *et al.*, 2009), and altered metabolism in specific brain regions (Eidelberg *et al.*, 1998). Electrophysiological analyses have also identified genotype-associated abnormalities, namely reduced intracortical inhibition and a shortened cortical silent period (Edwards *et al.*, 2003) as well as higher tactile and visuo-tactile temporal discrimination thresholds and temporal order judgments (Fiorio *et al.*, 2007). These subtle abnormalities in brain physiology, together with findings of no apparent neurodegeneration in the brains of affected DYT1 patients (Hedreen *et al.*, 1988; McNaught *et al.*, 2004; Rostasy *et al.*, 2003), suggest that neurodevelopmental differences in brain circuitry in *TOR1A* mutant gene carriers may underlie susceptibility to the dystonic phenotype. Factors contributing to penetrance are presumed to involve co-inheritance of genetic variants or environmental insults including drug exposure, peripheral injury, and viral infection (Edwards *et al.*, 2003; Saunders-Pullman *et al.*, 2004). Although none of these environmental causes have been proven, genetic modifiers, and specifically a variant in the *TOR1A* gene, D216H, which alternatively codes for aspartic acid (D) or histidine (H), has been associated with penetrance (Risch *et al.*, 2007).

In cellular models, when the 216H allele is overexpressed, ER/NE membrane inclusions are observed, similar to those seen when mutant torsinA is overexpressed (Kock *et al.*, 2006). However, when the H allele is co-overexpressed with a construct carrying the GAG deleted torsinA, fewer inclusions are formed than observed with GAG deletion only. This suggests that the two alleles jointly have a canceling effect. The D216H variant was studied in DYT1 families to assess its effect on penetrance. One hundred nineteen GAG deletion carriers "manifesting" dystonia were assessed, along with 113 "nonmanifesting" carriers and 197 controls. There was an increased frequency of the 216H allele in nonmanifesting

deletion carriers and a decreased frequency in manifesting carriers compared to the controls (Risch *et al.*, 2007). Analysis of haplotypes demonstrated a highly protective effect of the H allele when in trans with the GAG deletion, while evidence suggested that the D216 allele in cis was required for disease penetrance. These results, confirmed in one study, support the D216H variant as a potent intragenic modifier (Kamm *et al.*, 2008). Functional confirmation of this genetic association was observed in FDG PET imaging studies that showed differential effects of the D216H SNP in DYT1 nonmanifesting carriers (Carbon and Eidelberg, 2009).

Since the H allele is uncommon, present in only about 12% of the population, it only explains a small proportion of the reduced penetrance. Whether the D216H single nucleotide polymorphism alone plays a causative role in focal dystonia has also been investigated. One study found an association in various forms of familial focal/segmental dystonias (Brüggemann *et al.*, 2009); other studies of primarily sporadic cases however have failed to find an association (Kamm *et al.*, 2006; Sibbing *et al.*, 2003).

### 2. *DYT6 Dystonia*

DYT6 maps to chromosome 8p and mutations are inherited in an autosomal dominant pattern with reduced penetrance (Almasy *et al.*, 1997). The gene for DYT6, *THAP1*, was first identified in Amish Mennonite families (Fuchs *et al.*, 2009), whose causative mutation is a 5-base pair (GGGTT) insertion followed by a 3-base pair deletion (AAC) (c.135_139delinsGGGTTTA) in exon 2. This mutation results in a frame shift at amino acid 44 and a premature stop codon at position 73. *THAP1* mutations as a cause of PTD were initially thought to be restricted to related Amish Mennonite families, but different *THAP1* mutations in families with diverse ancestries have now been identified (Bonetti *et al.*, 2009; Bressman *et al.*, 2009; Djarmati *et al.*, 2009; Houlden *et al.*, 2010; Schneider *et al.*, 2009). In a clinically restricted group of non-DYT1 families with early-onset nonfocal PTD, 25% were found to have a *THAP1* mutation (Bressman *et al.*, 2009; Djarmati *et al.*, 2009; Schneider *et al.*, 2009). However, only 4.5% of a Dutch dystonia cohort with early-onset PTD and 2.5% of a British dystonia cohort with a variety of phenotypes were found to have *THAP1* mutations (Groen *et al.*, 2010; Ritz *et al.*, 2010). In addition to generalized dystonia, a small number of adult-onset focal cervical and laryngeal dystonia patients were found to have *THAP1* single base-pair substitutions (Xiao *et al.*, 2010).

Despite the diversity of mutations and ancestries, there is phenotypic similarity among cases. DYT6 dystonia usually begins in an arm, the neck, or other cranial muscles with two thirds eventually having involvement of the cranial muscles and impairment of speech. Symptom onset is typically early, but a significant proportion has symptoms begin after age 18 years.

*THAP1* contains a highly conserved THAP domain at the N-terminus and a nuclear localization domain at its C-terminus. THAP domains are atypical zinc fingers responsible for sequence-specific DNA binding, and THAP1 regulates many proteins involved in endothelial cell proliferation (Cayrol *et al.*, 2007). Two recent studies demonstrated that THAP1 also interacts with the *TOR1A* promoter region. Thus, *THAP1* mutations may lead to dystonia by transcriptional dysregulation of *TOR1A*. This interaction suggests that DYT1 and DYT6 dystonia share a common pathogenetic pathway (Gavarini *et al.*, 2010; Kaiser *et al.*, 2010).

3. *Other Generalized Primary Torsion Dystonias (DYT2, DYT4, DYT13, DYT17)*

In addition to DYT1 and DYT6, four other loci associated with early-onset or nonfocal PTD have been described. An autosomal recessive form of dystonia, DYT2, phenotypically resembling DYT1 dystonia, has been reported in five consanguineous families: three Spanish gypsy families (Gimenez-Roldan *et al.*, 1988), a Sephardic Jewish family (Khan *et al.*, 2003a, 2003b), and a family of Arabic descent (Moretti *et al.*, 2005). Dystonia presented early in all cases, typically beginning in the limbs and rapidly generalizing. To date, no linkage or mapping studies have been reported in these families.

In 1985, DYT4 dystonia was described in 20 members of a single large Australian family (Ahmad *et al.*, 1993; Parker, 1985). Dystonia typically began in the third decade and was focal to generalized with prominent whispering dysphonia. Disease transmission followed an autosomal dominant pattern with reduced penetrance. Linkage to a chromosomal locus has not been established.

The DYT13 locus (chromosome 1p36) was reported in a single large Italian family with early-onset autosomal dominant transmission with reduced penetrance (Valente *et al.*, 2001). Most cases had early onset (mean 16 years, range 5–43 years); the distribution of dystonia usually remained segmental with prominent craniocervical involvement; but 2 of 11 individuals also had mild leg dystonia (Bentivoglio *et al.*, 2004). Unlike DYT6, less speech involvement was reported. At present, no other families have been linked to this locus.

DYT17, an autosomal recessive form of PTD, was mapped to chromosome 20 in three affected siblings from a consanguineous Lebanese family (Chouery *et al.*, 2008). Dystonia began in adolescence with cervical muscle involvement, and like DYT6, progression was associated with severe dysphonia and dysarthria.

4. *Late-onset focal and segmental PTD (DYT7)*

Late- or adult-onset PTD, compared to early-onset PTD, is more likely to start in cervical or cranial muscles; the arm also is a common site of PTD onset in adults as it is in children. However, unlike early-onset PTD, late-onset PTD, regardless of muscles first involved, is much less likely to generalize. Based on the increased risk

of PTD observed in first degree relatives of individuals with focal PTD, autosomal dominant inheritance with reduced penetrance (about 12–15% compared to 30% for early-onset) has been proposed (Defazio *et al.*, 1993; Waddy *et al.*, 1991). Linkage studies in a large family from northwest Germany with seven affected members resulted in the mapping of the DYT7 locus to chromosome 18p (Leube *et al.*, 1996). Most affected members had adult-onset cervical dystonia (mean 43 years; range 28–70 years) although some also had brachial and cranial involvement. Three Bulgarian brothers with late-onset writer's cramp also mapped to chromosome 18p (Bhidayasiri *et al.*, 2005). Other clinically similar families have been excluded from linkage to DYT7, suggesting that there are other yet unidentified loci for adult-onset focal PTD (Jarman *et al.*, 1999).

Because the majority of adult-onset dystonia patients do not have affected relatives, association studies using cases and controls have been employed to find genetic risk factors. This method has been used to investigate the role of D5 dopamine receptor (*DRD5*), brain-derived neurotrophic factor (*BDNF*), *THAP1* (DYT6), and *TOR1A* (DYT1) in adult-onset dystonia. Since dopamine signaling is thought to be impaired in at least some forms of dystonia, several studies assessed a polymorphism in *DRD5*: two studies found an association between this polymorphism and adult-onset torticollis and blepharospasm (Misbahuddin *et al.*, 2002; Placzek *et al.*, 2001). V66M, a functional SNP in *BDNF*, has been shown to modulate human cortical plasticity (Cheeran *et al.*, 2008), and abnormal synaptic plasticity has been implicated in the pathogenesis of dystonia (Quartarone *et al.*, 2008). Investigations into whether the V66M SNP is associated with dystonia have reported contradictory results: one group found no association in a cohort of Italian patients with cranial and cervical dystonia (Martino *et al.*, 2009); and a second team found a two fold increase in V66M heterozygotes among cervical dystonia patients (Cramer *et al.*, 2010).

A number of studies have examined the influence of various SNPs in the PTD genes *TOR1A* and *THAP1* on the development of adult-onset PTD. Several non-coding SNPs in *THAP1* were identified in screening studies that included focal/segmental PTD patients, suggesting these variants might contribute to risk of focal dystonia (Djarmati *et al.*, 2009; Houlden *et al.*, 2010; Schneider *et al.*, 2009; Xiao *et al.*, 2010). However, the associated variants identified in these studies were not consistent between studies.

There has been more extensive examination of the *TOR1A* gene. As described above, results from association studies of the D216H SNP with dystonia have conflicted, which may reflect differences in case ascertainment. Several SNPs from the 3′ untranslated region of TOR1A have been implicated in focal dystonia in Icelandic and Italian patients (Clarimon *et al.*, 2005; Clarimon *et al.*, 2007) and in the risk of spread of blepharospasm in Italian and US cohorts (Defazio *et al.*, 2009). Two other studies, one in an Austrian and German population with focal dystonia (Kamm *et al.*, 2006) and the other in a mixed European cohort with focal and segmental dystonia (Sharma *et al.*, 2010), showed an association with 3′ SNPs, but

in both cases, rather than being a risk haplotype, the SNPs showed a strong protective effect. Finally, two studies from Germany failed to show any association between SNPs in the TOR1A gene and focal dystonia (Hague *et al.*, 2006; Sibbing *et al.*, 2003). Thus, the results of association studies remain inconclusive. This may be due in part to limited sample size and the clinical and ethnic heterogeneity among PTD patients. Genome wide association studies of large clinically homogeneous groups or next generation analyses of select families or subtypes are needed to clarify genetic risk factors for late-onset PTD.

## II. Dystonia-Plus Syndromes without Brain Degeneration

Neither dystonia-plus syndromes nor PTD are associated with brain degeneration. Dystonia-plus syndromes are distinguished by having clinical features in addition to dystonia, specifically parkinsonism and myoclonus. This group includes four discrete disorders, dopa-responsive dystonia (*DYT5, DYT14*), myoclonus-dystonia (DYT11, DYT15), rapid-onset dystonia-parkinsonism (DYT12), and dystonia-parkinsonism (DYT16).

### 1. *Dopa-responsive dystonia (DYT5, DYT14)*

Typically, dopa-responsive dystonia (DRD) begins in childhood with gait abnormalities. Dystonia is often diurnal, worsening as the day progresses and improving after sleep. Arm dystonia, hyperreflexia, and parkinsonism – bradykinesia, hypomimia, and postural instability – are also common features (Nygaard *et al.*, 1992). In one recent series, dystonia also involved cervical and cranial muscles, including the upper face, lower face, and larynx (Trender-Gerhard *et al.*, 2009). Over the years the clinical spectrum of this disorder has been broadened to include adult-onset parkinsonism, action tremor, psychiatric features of major depression, obsessive compulsive disorder, sleep disorders (Van Hove *et al.*, 2006), adult-onset oromandibular dystonia (Steinberger *et al.*, 1999), developmental delay with spasticity mimicking cerebral palsy (Nygaard *et al.*, 1994), scoliosis (Furukawa *et al.*, 2000), and generalized hypotonia with proximal weakness (Kong *et al.*, 2001). The hallmark of all clinical subtypes of DRD is a dramatic and sustained response to low-dosage levodopa although rarely the dosage required may be substantial, especially in those with compound heterozygous mutations or symptom onset during adulthood.

The genetic cause for the majority of cases of DRD is an autosomal dominantly inherited mutation in GTP cyclohydrolase 1 (*GCH1*) (Hagenah *et al.*, 2005; Ichinose *et al.*, 1994; Ichinose *et al.*, 1999). *GCH1* is the first and rate-limiting enzyme in the synthesis of tetrahydrobiopterin, an essential cofactor for tyrosine

hydroxylase (TH), the enzyme that converts tyrosine to L-dopa (Ichinose *et al.*, 1999). New mutations appear to occur commonly, and some mutations are deletions not detectable by qualitative screening. There is reduced penetrance in examined families, and for unclear reasons, women are more likely to manifest symptoms than men (Furukawa *et al.*, 1998).

In addition to heterozygous *GCH1* mutations, DRD may be caused by compound heterozygous mutations in *GCH1* and homozygous, compound heterozygous, and heterozygous mutations in other enzymes involved in dopamine synthesis. The genes for TH (DYT14) (van den Heuvel *et al.*, 1998), 6-pyruvoyltetrahydropterin synthase (Hanihara *et al.*, 1997), and sepiapterin reductase (Clot *et al.*, 2009) have all been implicated in DRD. Unlike DRD due to mutations in *GCH1*, these genetic forms of DRD often have a more complex phenotype that includes mental retardation, oculogyria, hypotonia, severe bradykinesia, drooling, ptosis, miosis, and seizures (Clot *et al.*, 2009; Steinberger *et al.*, 2004). However, symptoms may be mild and limited to typical DRD signs, mild spastic paraplegia, or exercise-induced stiffness which resolve with administration of levodopa (Furukawa *et al.*, 2001). Individuals with homozygous *PRKN* mutations may present with a syndrome that mimics DRD (Khan *et al.*, 2003a, 2003b; Tassin *et al.*, 2000). Although these patients will respond similarly to levodopa, they can usually be differentiated from DRD by early parkinsonism and significant medication-induced dyskinesia.

2. *Myoclonus–Dystonia (DYT11, DYT15)*

It was recognized in early descriptions of dystonia that some patients have jerks of short duration, about 100 ms, which resemble myoclonus. This may occur in DYT1 and other forms of primary dystonia (Obeso *et al.*, 1983), but it may also be the only movement disorder (Mahloudji and Pikielny, 1967). In families with myoclonus-dystonia (M-D), affected individuals have myoclonus with or without dystonia, which is usually mild. Rarely dystonia is the only feature (Kyllerman *et al.*, 1990). The myoclonus may be isolated jerks at rest as well as complex oscillatory or pseudo-rhythmic bursts, especially as an overflow phenomenon. Neurophysiological studies support a subcortical origin for the myoclonus (Marelli *et al.*, 2008).

Symptom onset is typically in the first or second decade, and the disorder tends to plateau in adulthood. The neck and arms are most commonly involved, followed by the trunk and bulbar muscles. The legs are less commonly involved. Affected adults often report that muscle jerks respond dramatically to alcohol. An excess of psychiatric symptoms, anxiety, depression, panic attacks, and OCD, has been reported in family members, even those not affected with motor signs of M-D (Foncke *et al.*, 2009; Hess *et al.*, 2007).

In familial M-D, the pattern of inheritance is autosomal dominant with reduced penetrance. An M-D locus on chromosome 7q21 (DYT11) was first

mapped in a North American family with 10 affected individuals with typical M-D clinical features (Nygaard *et al.*, 1999). In 2001, the gene was identified as epsilon-sarcoglycan (*SGCE*) based on different loss of function mutations in six families (Clarimon *et al.*, 2007). Large exon deletions have been shown to account for some of the cases originally thought to be mutation-negative (Asmus *et al.*, 2007; Grunewald *et al.*, 2008). In patients with heterozygous deletions of the entire *SGCE* gene, the deletion may also involve adjacent genes accounting for additional characteristics, such as skeletal abnormalities due to a deletion of the neighboring *COL1A2* gene.

*SGCE* is maternally imprinted via methylation of CpG sites in the promoter region, which effectively silences the maternal allele. Thus, clinically evident M-D is usually inherited from the father, who may or may not be affected depending on the sex of his transmitting parent (Grabowski *et al.*, 2003). However, in 10% of M-D patients, penetrance does not follow the expected pattern. RNA expression studies have revealed expression of only the mutated allele in affected individuals and expression of the normal allele in unaffected mutation carriers (Grabowski *et al.*, 2003; Muller *et al.*, 2002). In one M-D patient, partial loss of methylation at several CpG dinucleotides, was shown to be associated with bi-allelic expression of the *SGCE* gene (Muller *et al.*, 2002). Maternal uniparental disomy, resulting in two silenced *SGCE* alleles, has been identified as a cause of M-D and coincident Russell–Silver Syndrome (Guettard *et al.*, 2008).

Alpha, beta, gamma, and delta sarcoglycan are all components of the dystrophin–glycoprotein complex. Homozygous or compound heterozygous mutations in these sarcoglycans result in limb-girdle dystrophy. The normal function of epsilon sacrcoglycan remains unknown. It is widely expressed in neurons where it localizes to the plasma membrane. A recent study characterizing different SGCE isoforms in the human brain found that that a major brain-specific isoform was differentially expressed: there was high expression in the cerebellar Purkinje cells and neurons of the dentate nucleus, low expression in the globus pallidus, and moderate to low expression in the caudate nucleus, putamen, and substantia nigra. This suggests that the primary area of dysfunction in M-D is in the cerebellum (Ritz, *et al.*, 2010). Interestingly a KO mouse model demonstrated alteration in brain levels of dopamine, dopamine metabolites, and serotonin metabolites (Dang *et al.*, 2006; Yokoi).

*SGCE* mutations do not account for all familial M-D and probably are not responsible for most sporadic M-D. The proportion of M-D and clinically related phenotypes due to *SGCE* mutations is debated and depends on the clinical criteria used to select subjects for screening (Ritz *et al.*, 2009). A second M-D locus, 18p11 (DYT15), was mapped in a large Canadian family in 2002. The responsible gene has not yet been identified (Grimes *et al.*, 2002; Han *et al.*, 2007).

3. *Rapid-onset dystonia-parkinsonism (DYT12)*

Rapid-onset dystonia-parkinsonism (RDP) is a rare dystonia-plus syndrome that starts in childhood or early adulthood. It is characterized by the emergence of dystonia and parkinsonism over hours to days. However, symptoms may begin insidiously and later be followed by a period of rapid worsening. After the period of acute worsening, symptoms tend to stabilize and improvement may even occur (Brashear *et al.*, 2007; McKeon *et al.*, 2007). The phenotype resembles the dystonia and parkinsonism of Wilson's disease with prominent bulbar signs (including risus sardonicus), sustained dystonic limb posturing, waxy effortful rapid successive movements, and postural instability.

Inheritance is autosomal dominant but *de novo* mutations have been observed. The responsible gene, *ATP1A3*, maps to chromosome 19q13 (DYT12) and codes for $Na^+/K^+$-ATPase alpha3, a catalytic subunit of the sodium-potassium pump (de Carvalho Aguiar *et al.*, 2004). Missense mutations, the only type currently identified, result in dysfunctional $Na^+/K^+$-ATPase and reduced cellular ion transport. The ability of the haploinsufficient cell to compensate may be overwhelmed during times of physiological stress.

4. *Dystonia-parkinsonism (DYT16)*

Dystonia-parkinsonism (DYT16) was first described in three Brazilian families. Affected individuals exhibited early-onset dystonia with generalization, and similar to DYT6, they had prominent bulbar involvement with dysphonia, dysarthria, and dysphagia. Four of the seven exhibited parkinsonism. The syndrome was attributed to homozygous missense mutations (P222L) in *PRKRA* (protein kinase, interferon-inducible double-stranded RNA-dependent activator) on chromosome 2q31.2 (Camargos *et al.*, 2008). Subsequently, a patient with early-onset generalized dystonia was reported to have a heterozygous frameshift mutation in *PRKRA* (*Seibler et al.*, 2008). Although the first cases were recessively inherited, the subsequent case suggests that heterozygous mutations may produce disease. Little is known about the function of the PRKRA protein.

## III. Dystonia as a Feature of Degenerative Genetic Syndromes

There are many neurodegenerative genetic diseases with dystonia as a common feature. (Table II) These include X-linked dystonia-parkinsonism (DYT3), which is discussed below, and two rare recessively inherited causes of dystonia-parkinsonism in which parkinsonism is a dominant feature. The loci are

Table II
Neurodegenerative Inherited Diseases with Dystonia as a Feature of Disease.

| | |
|---|---|
| Autosomal dominant | Huntington's disease (HD) |
| | Machado-Joseph's disease/SCA3 disease |
| | Other SCA subtypes (e.g. SCA 2, 6, 17) |
| | Familial basal ganglia calcifications (Fahr's) |
| | DRPLA |
| | Neuroferritinopathy |
| Autosomal recessive | Juvenile parkinsonism (Parkin) |
| | Wilson's |
| | Glutaric acidemia |
| | PKAN/Hallervorden–Spatz disease |
| | Lysosomal diseases (GM1, GM2, Neimann Pick type C (NPC1), metachromatic leukodystrophy, ceroid–lipofuscinosis) |
| | Homocystinuria |
| | Propionic acidemia |
| | Methylmalonic aciduria |
| | Ataxia–telangiectasia (AT) |
| | Ataxia with Vitamin E Deficiency |
| | Recessive Ataxia with Ocular Apraxia (AOA1, AOA2) |
| | Neuroacanthocytosis |
| | Neuronal intranuclear inclusion disease (NIID), |
| | Hemachromatosis |
| | Aceruloplasminemia |
| X-linked recessive | Lubag (X-linked dystonia-parkinsonism, *DYT3*) |
| | Lesch–Nyhan |
| | Deafness/Dystonia Syndrome |
| | Pelizaeus-Merzbacher Disease |
| Mitochondrial | MERRF/MELAS |
| | Leber's disease |

designated PARK14 and PARK15, corresponding to the genes *PLA2G6* and *FBXO7*, respectively (Schneider *et al.* 2009).

1. *X-linked dystonia-parkinsonism (DYT3)*

Lubag disease is an adult-onset X-linked form of dystonia and parkinsonism observed among Filipino males, primarily from the island of Panay. Affected individuals can present with either dystonia or parkinsonism but all patients eventually develop parkinsonism (Lee *et al.*, 1976). Isolated parkinsonism is considered a more benign phenotype (Evidente *et al.*, 2002a, 2002b). Neuropathological studies show degeneration and gliosis of the caudate and lateral putamen (Waters *et al.*, 1993a, 1993b). The phenotypic spectrum has been broadened to include tremor, myoclonus, chorea, and myorhythmia (Evidente *et al.*,

2002a, 2002b). Likewise, heterozygous females have been reported who have mild dystonia or chorea (Evidente *et al.*, 2004; Waters *et al.*, 1993a, b).

Owing to a founder effect, all cases of Lubag disease are attributed to a retroposon insertion in one of the introns of *TAF1* at Xq13.1, resulting in decreased expression of the *TAF1* transcript in the caudate (Makino *et al.*, 2007). Three Italian siblings with early-onset dystonia and a shared haplotype at the DYT3 locus, different from the Filipino haplotype, indicate a possible second causative mutation. (Fabbrini *et al.*, 2005). How mutant *TAF1* leads to dystonia and parkinsonism is unknown.

## IV. Treatment of Dystonia

Treatment for most forms of dystonia is empiric and directed toward improvement of dystonic movements, daily function, pain relief, and psychiatric symptoms if present. There are currently no gene-directed therapies; however, several dystonia subtypes have effective treatments that target underlying causative mechanisms. These include DRD (discussed below), Wilson's disease, and DYT18 dystonia due to GLUT1 deficiency. Aside from these disorders, main dystonia treatment options consist of oral medications, pallidal deep brain stimulation (DBS) and botulinum toxin injections. Application of the latter two approaches over the last 20 years has led to tremendous progress in improving symptoms of dystonia.

Oral medications are still commonly used in childhood and adolescent-onset dystonia especially when there is widespread muscle involvement. Multiple medications with different mechanisms of action are usually required to achieve improvement in symptoms. Objective data regarding treatment response is limited by the heterogeneity of clinical expression, often unknown etiologies, limited sample size, and imperfect outcome measures. Indeed, there is very little placebo-controlled data to support pharmacological treatment.

### A. Pharmacological Treatment

#### 1. *Dopaminergic and antidopaminergic drugs*

To exclude DRD, all patients with early onset dystonia should receive a trial of levodopa combined with carbidopa for up to 1 month. Most patients with DRD respond dramatically to low doses of levodopa (100–300 mg of levodopa per day combined with carbidopa in divided doses) and do not develop a fluctuating response or dyskinesias. Modest improvement with levodopa therapy has also

been reported in some patients with other types of dystonia, including myoclonus-dystonia (Luciano *et al.*, 2009) and secondary dystonia (Bernard *et al.*, 2010; Head *et al.*, 2004; Neubauer *et al.*, 2005). In addition to levodopa, patients with DRD may also receive benefit from anticholinergic medications (Jarman *et al.*, 1997), dopamine agonists, and carbamazepine.

Because of limited effectiveness and the possibility of drug-induced parkinsonism and tardive dyskinesia, neuroleptic drugs are not commonly used for the treatment of primary dystonia. Clozapine, an atypical neuroleptic not associated with drug-induced parkinsonism or tardive syndromes, was shown to provide moderate benefit for segmental and generalized dystonia in a small, open-label trial (Karp *et al.*, 1999). However, its usefulness is limited by the potential serious side effect of agranulocytosis.

Tetrabenazine has proven beneficial in some patients with dystonia, in particular those with tardive dystonia (Kenney *et al.*, 2007), and it is now approved in the United States for treatment of chorea in Huntington's disease. As an inhibitor of vesicular monoamine transporter 2, it does not cause tardive dyskinesia but can cause transient acute dystonic reaction, parkinsonism, and depression (Kenney and Jankovic, 2006).

2. *Anticholinergic drugs and muscle relaxants*

Anticholinergic drugs, such as trihexyphenidyl, are widely used in the treatment of segmental and generalized dystonia (Burke *et al.*, 1986; Greene *et al.*, 1988). They are usually tolerated in children and are the pharmacological treatment of choice for childhood-onset generalized PTD. Initial doses should be low and increased slowly to minimize sedation, confusion, memory difficulty, and hallucinations, which may occur at higher doses. Target doses can range from 25 to 45 mg a day, depending on age and weight, and doses up to 60 to 100 mg a day have been used. Significant peripheral anticholinergic side effects can usually be mitigated with small doses of an acetylcholinesterase inhibitor such as pyridostigmine.

Baclofen is a presynaptic GABA-receptor agonist that can be administered orally or intrathecally in severe cases of spastic dystonia with good results (Woon *et al.*, 2007). Cyclobenzaprine and tizanidine have been used with variable success. Benzodiazepines, such as diazepam, lorazepam and clonazepam are often used for their muscle-relaxant properties. Clonazepam is especially useful in blepharospasm and M-D (Zimprich *et al.*, 2001).

3. *Other pharmacological treatments*

Sodium oxybate (Xyrem) has been shown to improve myoclonus in alcohol-responsive M-D in a single-blind, open-label trial (Frucht *et al.*, 2005).

There are several other agents that have been tried with varying results for alleviating symptoms of dystonia, including morphine sulfate and mexiletine. Due to side effects and limited efficacy, these treatments are rarely used today. There are isolated reports that zolpidem might be helpful in certain types of dystonia (Evidente, 2002). A number of antiepileptic drugs have been used in the treatment of dystonia. While there are case reports of benefit from topiramate and levetiracetam, (Papapetropoulos and Singer, 2006; Sullivan *et al.*, 2005; Zesiewicz *et al.*, 2004), there have been no randomized studies or large case series.

4. *Dystonic Storm and its treatment*

Rarely, patients can experience "dystonic storm," or dystonic status, with severe dystonia and extreme posturing that may compromise breathing or cause life-threatening hyperthermia and rhabdomyolysis (Manji *et al.*, 1998). Dystonic storm may represent the natural course of severe dystonia or be triggered by infection or drug withdrawal (Manji *et al.*, 1998; Mariotti *et al.*, 2007). Therapeutic intervention should be prompt and usually requires admission to an intensive care unit. One algorithm suggests initial treatment with intravenous benzodiazepine and anesthetic agents; GPi deep brain stimulation and intrathecal baclofen pump may be required in refractory cases (Mariotti *et al.*, 2007).

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# EXPERIMENTAL MODELS OF DYSTONIA

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Dystonia is a disabling movement disorder characterized by involuntary, sustained muscle contractions, with repetitive twisting movements and abnormal postures. It is clinically classified as primary, either sporadic or genetic, or secondary, following focal brain lesions. The recent past has witnessed remarkable progress in finding genes for dystonia. However, translating the findings from genetics into concrete changes for dystonic patients is not immediate, as it requires extensive exploration of the consequences of gene defects on motor behavior, protein biochemistry, and cell physiology. Thus, in the last decade, a number of animal models have been generated and, to some extent, characterized. These include distinct species, ranging from invertebrates, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, to rodents and nonhuman primates. The mouse is the average choice of mammalian models in most laboratories, particularly when manipulations of the genome are planned. Investigations of animals provide results that do not always reproduce the clinical features of human dystonia. Indeed, most of the mouse models of inherited dystonia do not exhibit overt dystonia although they do have subtle motor abnormalities and well-characterized neurochemical and neurophysiological alterations. Conversely, spontaneous mutant models display a clear phenotype, but in some cases the origin of the mutation is unknown. In spite of such limitations and apparent contradictory evidence, there is general consensus on the notion that a useful animal model has to be judged by how reliably and effectively it can be used to explore novel aspects of pathophysiology and potential treatments. In the present work, we briefly describe the most commonly utilized models for the study of dystonia and the results obtained, in attempt to provide a comprehensive overview of the current, available models.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00020-1




0074-7742/10 $35.00

## I. Introduction

The dystonias are a clinically and genetically heterogeneous group of movement disorders, characterized by involuntary muscle contractions that cause repetitive movements and/or abnormal postures (Fahn, 1988). These motor deficits may be the sole clinical manifestation or occur as secondary symptoms due to other underlying disease processes.

Advances in neurogenetics have allowed the identification of ~20 forms of inherited dystonia associated with specific mutations, but the mechanisms by which these mutations lead to disease are not entirely clear (Brüggemann and Klein, 2010). The lack of any characteristic neuropathology led to hypothesize that the pathophysiology of dystonia can be envisioned as a multistep pathway that begins with a "trigger" event or risk factor that induces multiple downstream consequences and ultimately alters motor system resulting in symptom generation. More recently, data obtained from imaging studies suggest to define dystonia as a neurodevelopmental circuit disorder, highlighting the relevance of an underlying network distortion (Argyelan *et al.*, 2009).

In the past decade, there has been much effort in the development of animal models, and particularly rodent models of inherited dystonia (LeDoux, 2010; Raike *et al.*, 2005), in attempt to elucidate its pathogenesis, and to facilitate the discovery of potential novel treatments. Multiple animal models for dystonia have now been generated and characterized. Studies of these models have produced experimental data that, to some extent, lead in different directions, in part because the different models target distinct aspects of a very heterogeneous disorder (Breakefield *et al.*, 2008; Tanabe *et al.*, 2009). On the other hand, clinical and experimental observations converge to suggest the existence of some common pathogenic features, such as the involvement of basal ganglia and the prominent role of dopamine signaling (Augood *et al.*, 2004; Perlmutter and Mink, 2004; Pisani *et al.*, 2006; Wichmann, 2008; Zhao *et al.*, 2008).

The selection of a suitable animal model is complex, and many factors should be considered, such as (1) convenience, (2) appropriateness, (3) transferability of information, (4) genetic uniformity of organisms where applicable, (5) background knowledge of biological properties, (6) adaptability to experimental manipulation, and (7) ethical considerations (Davidson *et al.*, 1987). It is implicit that the available models may meet some but certainly not all of the considerations set. Although rodents may be considered less attractive than nonhuman primates as models for human disease, it is comprehensible that, for a number of reasons, the rodent models offer opportunities that are not realistic with other species.

In the present review, we tried to concisely and critically analyze the most commonly utilized models for the study of dystonia. Although there may be different ways of classifying these models, here they were categorized into: (i) genetic models; (ii) spontaneous mutants; and (iii) pharmacological and neural lesion models.

## II. Models of Genetic Engineering

### A. Invertebrates

*Drosophila melanogaster* and *Caenorhabditis elegans* are extremely simple organisms and they represent a reliable tool to study the function of specific genes. Many advantages can result from the use of invertebrates as a substrate for the creation of genetic models of human disease. These organisms reproduce quickly and in large numbers, and can be easily reared in the laboratory. Moreover, they possess a very simple and completely sequenced DNA, and gene mutation can be produced artificially or may appear spontaneously (C. Elegans Sequencing Consortium, 1998). These simple organisms have also different molecular pathways conserved during the evolution process. For example, *C. elegans* can be used to study the mechanisms involved in the packaging, processing, and transport of proteins (Harrington *et al.*, 2010).

On such basis, some invertebrate models of dystonia have been generated, with a specific focus on DYT1 dystonia (Table I). A 3bp mutation of the protein torsinA causes early-onset DYT1 dystonia in humans although the precise mechanism by which this genetic alteration leads to the onset of the disease is to date

Table I
Invertebrate Models[a].

| Disorder(s) | Protein | Model | Results | References |
|---|---|---|---|---|
| DYT1 | torsinA | *C. elegans*-transgenic overexpression of human TA | Reduction in polyglutamine repeat-induced protein aggregation | Caldwell *et al.*, 2003 |
| | | *C. elegans*-transgenic overexpression of ΔE human TA/WT human TA | TA reduces stress RE and maintaining cellular homeostasis | Chen *et al.*, 2010 |
| | | | Identify two classes of antibiotics, which enhance the properties of WT TA | Cao *et al.*, 2010 |
| DYT1 | torsinA | Drosophila-transgenic overexpression ΔE human TA | Enlarged boutons neuromuscular junction, abnormality of TGF-$\beta$ signaling | Koh *et al.*, 2004 |
| DYT1 | torsinA | Drosophila-RNAi downregulation of torp4A | Progressive retinal degeneration | Muraro & Moffat, 2006 |

[a] Abbreviations: WT, wild-type; ER, endoplasmic reticulum; TA, torsinA; ΔE TA, mutant torsinA;

unclear (Ozelius *et al.*, 1997). Herein, we will refer to this mutant protein as ΔETorsinA. TorsinA belongs to the AAA+ (ATPases associated with a variety of cellular activities) superfamily of chaperone-like proteins, assisting in protein trafficking, membrane fusion, and participating in secretory processing (Goodchild *et al.*, 2005; Granata *et al.*, 2008; Hewett *et al.*, 2007; Tanabe *et al.*, 2009). Recent evidence suggests that torsinA disassembles protein complexes or otherwise changes the conformation of proteins in the endoplasmic reticulum as well as in the nuclear envelope (Chen *et al.*, 2010; Tanabe *et al.*, 2009).

In contrast to mammals, which express four members of the torsin superfamily, *D. melanogaster* contains only a single member based on homology in the genome sequence (Ozelius *et al.*, 1999). In *D. melanogaster.*, the homologue gene is called *torp4a* and shows a 34% homology to human torsinA (Muraro and Moffat, 2006). By means of RNA interference (RNAi), these authors demonstrated that down-regulation of torp4a caused degeneration of the retina, whereas its over-expression was able to protect the retina from age-related neural degeneration, a phenomenon which is known to be dependent on lysosome activity. These results were indicative of a role of *torp4a* in transport and function of lysosome-related organelles (Muraro and Moffat, 2006) (Table I).

In addition, expression of human ΔETorsinA in *D. melanogaster* was reported to cause abnormal motor behaviors in the fly (Koh *et al.*, 2004) (Table I).

Three torsin-related genes OOC-5, Y37A1B.12, Y37A1B.13 have been identified in the nematode model organism *C. elegans* (Ozelius *et al.*, 1999). Consistent with a role for torsinA in neuronal survival, overexpression of human ΔE torsinA in *C. elegans* was able to prevent 6-hydroxy-dopamine-induced degeneration in dopaminergic neurons (Cao *et al.*, 2005) and to protect COS-1 and PC-12 cells against oxidative stress (Kuner *et al.*, 2003).

By using such nematode model, Caldwell and coworkers investigated human mutations associated with dystonia for their functional impact on endoplasmic reticulum stress, a cellular response to aberrant protein trafficking and folding (Chen *et al.*, 2010). These data indicate that a normal function of torsinA is to prevent the onset of intracellular stress that ensues when this process is impaired, and that deficits in torsinA activity at luminal level, perhaps caused by mislocalization of torsinA to the nucleus, sensitizes cells to intracellular stress caused by protein misfolding at the endoplasmic reticulum (Chen *et al.*, 2010). Together, these findings suggest a functional role for torsinA in maintaining intracellular homeostasis that is altered in DYT1 dystonia.

### B. Vertebrates

Among vertebrates, the mouse is the most common species utilized for genetic manipulation (Metzger and Feil, 1999). Rodents, in fact, display several advantages

as compared to other vertebrate species. These animals reproduce in large numbers, and they are easy to breed with a relative low cost. Several genetic engineering mouse models are now available, particularly for the study of DYT1 dystonia. In some models, the human *TOR1A* gene is inserted randomly in the mouse genome, while in other lines the mouse locus *TOR1A* has been manipulated in different ways either through gene modification (knock-in, KI), gene expression reduction (knock-down, KD), or through the total gene inhibition (knock-out, KO) (Tables II and III).

Four transgenic mouse models have been created through over-expression of human mutated torsinA protein (ΔETorsinA), utilizing different promoters in the mouse genome (Grundmann *et al.*, 2007; Page *et al.*, 2010; Sharma *et al.*, 2005; Shashidharan *et al.*, 2005) (Table II). In these models, the quality of temporal and spatial transgene promoter expression depends on the design construct and the insertion site (Zhao *et al.*, 2008).

In the transgenic mouse generated by Shashidharan and coworkers (2005), human ΔETorsinA protein was overexpressed using a neuron-specific enolase (NSE) promoter. About 40% of these mice showed hyperactivity and abnormal movements. Conversely, Grundmann and collaborators developed a transgenic mouse using the murine prion protein promoter (Grundmann *et al.*, 2007). These animals showed impaired motor performance. Both models presented abnormal levels of dopamine (DA) metabolites (Grundmann *et al.*, 2007; Shashidharan *et al.*, 2005). Immunohistochemistry experiments also demonstrated some perinuclear inclusions and aggregates that stained positively for ubiquitin and torsinA (Grundmann *et al.*, 2007; Shashidharan *et al.*, 2005). In addition, autoradiographic studies showed a decreased density of striatal D2 DA receptors, with no difference in D1 receptors or DA transporter (DAT) binding in transgenic mice over-expressing human ΔETorsinA under the NSE promoter (Giannakopoulou *et al.*, 2010).

In the transgenic mouse developed by Sharma and colleagues (2005), human mutant torsinA was over-expressed under a cytomegalovirus promoter (CMV). These mice showed no overt dystonic behavior, but exhibited motor learning impairments (Sharma *et al.*, 2005; Zhao *et al.*, 2008). No cytoplasmic torsinA and ubiquitin-positive inclusions were found in this model (Zhao *et al.*, 2008). In addition, these mice did not show any alteration of basal striatal DA level, but showed a significantly reduced amphetamine-evoked DA release (Balcioglu *et al.*, 2007), an effect that has been ascribed to a deficient DAT activity (Hewett *et al.*, 2010). Moreover, the concentration of DA metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) was altered, indicative of an increased DA turnover (Zhao *et al.*, 2008). Cell physiology has been extensively characterized in this same mouse model, and the experimental data collected suggest a profound alteration of both striatal dopaminergic and cholinergic systems. Most striatal neurons are medium spiny projection neurons (MSNs),

Table II
TRANSGENIC MODELS[A].

| Disorder(s) | Protein | Model | Results | References |
|---|---|---|---|---|
| DYT1 | torsinA | ΔE human TA under neuron-specific enolase (NSE) promoter | Hyperkinesias; self- clasping; rapid bi-directional circling; TA and ubiquitin positive inclusions in PPN, pons and PAG; decreased DA; decreased DOPAC/DA; | Shashidharan *et al.*, 2005 |
| | | | Peak single-pulse evoked extracellular DA concentration was significantly lower in phenotype-positive mice | Bao *et al.*, 2010 |
| | | | Decrease of D2-dopamine receptors | Giannakopoulou *et al.*, 2010 |
| DYT1 | torsinA | ΔE human TA/ WT human TA under cytomegalovirus immediate early promoter | ΔE mice have reduced learning motor skill on rotarod; no evidence of inclusions; | Sharma *et al.*, 2005 |
| | | | Striatal cholinergic interneurons in the presence of quinpirole show an increase of firing mediated by inhibition of N-type calcium currents | Pisani *et al.*, 2006 |
| | | | Impaired release of DA upon treatment with amphetamine; no difference in level of DA metabolites, DAT, VMAT2 and D1 D2 post-synaptic receptors. | Balcioglu *et al.*, 2007 |
| | | | ΔE mice with abnormal motor phenotype in raised-beam analyses; higher DOPAC/DA; HVA/DA; no difference in DA level | Zhao *et al.*, 2008 |
| | | | Altered GABAergic input onto striatal medium spiny neurons | Sciamanna *et al.*, 2009 |
| | | | Alterations of corticostriatal synaptic plasticity, with a significant increased LTP and the lost of LTD | Martella *et al.*, 2009 |
| | | | Normal responsiveness to D2-autoreceptor function in nigral dopaminergic neurons. Blockade of adenosine A2A receptors fully restored the impairment of synaptic plasticity observed in ΔE mice; reduction of striatal D2R protein, Haloperidol induce a reduced cataleptic response in ΔE mice | Napolitano *et al.*, 2010 |

(*continued*)

Table II (*continued*)

| Disorder(s) | Protein | Model | Results | References |
|---|---|---|---|---|
| | | | Altered DAT function | Hewett *et al.*, 2010 |
| DYT1 | torsinA | ΔE human TA/ WT human TA under murine prion protein promoter | Hyperactivity and defects on rotarod testing; TA laminin and ubiquitin positive inclusions in the brainstem, nuclear envelope bleb formation; decreased DA, and serotonin | Grundmann *et al.*, 2007 |

[a] Abbreviations: TA, torsinA; PPN, pedunculopontine nucleus ; PAG periaqueductal gray LTP, long term potentation; LTD, long-term depotentation; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; DAT, DA transporter; VMAT2, vesicular monoamine transporter protein.

constituting nearly 95% of the entire neuronal population. The remaining striatal neurons are interneurons. Among these, cholinergic interneurons are giant aspiny interneurons with richly arborizing axons with large terminal fields, leading to high striatal levels of markers of cholinergic signalling, such as

Table III
Other Genetic Engineering Models[A].

| Disorder(s) | Protein | Model | Results | References |
|---|---|---|---|---|
| DYT1 | torsinA | Dyt1 ΔGAG knockin | Deficiency on beam-walking test; hyperactivity; TA and ubiquitin positive inclusions in PPN. Decreased of HA | Dang *et al.*, 2005 |
| DYT1 | torsinA | Dyt1 ΔGAG knockin | Homozygous is lethal; NE abnormalities | Goodchild *et al.*, 2005 |
| DYT1 | torsinA | Dyt1 knockdown | Homozygous is lethal; NE abnormalities | Goodchild *et al.*, 2005 |
| DYT1 | torsinA | Dyt1 knockdown | Deficiency in beam-walking; hyperactivity; decreased DOPAC | Dang *et al.*, 2006 |
| DYT1 | torsinA | Cortex-specific Dyt1 conditional knockout mice | Deficiency on beam-walking test; hyperactive; no alteration in striatal DA | Yokoi *et al.*, 2008 |
| DYT1 | torsinA | TH promoter to direct transgene expression specifically to dopaminergic neurons | Basal hypoactivity; deficit beam walking test; striatal imbalanced DA release | Page *et al.*, 2010 |

[a] Abbreviations: PPN, pedunculopontine nucleus; NE, nuclear envelope; DOPAC, 3, 4-dihydroxyphenylacetic acid; TH, tyrosine hydroxylase; DA, dopamine.

choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and the vesicular acetylcholine transporter (VAChT) (Bolam *et al.*, 1984). Cholinergic interneurons are solely found in regions with a dense dopaminergic innervation, such as the dorsal striatum. In these regions, dopaminergic afferents exert a powerful control over cholinergic transmission. Several lines of experimental and clinical evidence point to the striatum, where DA and acetylcholine (ACh) interact, as a principal locus of the pathophysiological changes in brain function underlying dystonia (for rev. see Pisani *et al.*, 2007). Our studies identified a fundamental change in striatal cholinergic signaling. Striatal cholinergic interneurons exhibit autonomous pacemaker activity, providing a constant ACh tone in the striatum. Maintenance of ACh levels is regulated by ACh degrading enzymes, by muscarinic $M_2/M_4$ autoreceptors and by an inhibitory D2 receptor action. Normally, activation of DA D2 receptors reduces the activity of cholinergic interneurons (Pisani *et al.*, 2000). However, in interneurons from transgenic mice with the DYT1 mutation, D2 receptor activation dramatically increased, rather than decreased spike rate, thereby causing an elevation of ambient ACh (Pisani *et al.*, 2006). Of note, striatal MSNs exhibited significant abnormalities of long-term synaptic plasticity. Indeed, MSNs of these mice lack long-term depression (LTD) and synaptic depotentiation (SD), while conversely, they express an enhanced long-term potentiation (LTP) (Martella *et al.*, 2009). The inability to revert synaptic strength from the potentiated state to pre-LTP levels may indeed result in the loss of a "surround inhibition," and therefore the loss of the skill to select from competing motor patterns, which is consistent with the main pathogenic features of dystonic symptoms. Our results demonstrated that an enhanced cholinergic tone, through activation of muscarinic M1 receptors is responsible for corticostriatal plasticity deficits in mice with mutant torsinA. Both LTD and SD, in fact, were restored either by lowering striatal ACh with drugs preventing ACh resynthesis or by antagonizing M1 receptors (Martella *et al.*, 2009).

Rodent models have also been engineered to address the role of specific brain regions in DYT1 dystonia. Yokoi and coworkers (2008) developed cerebral cortex-specific DYT1 conditional KO mice. The conditional KO mice showed normal development of somatosensory cortex, hyperactivity and impairment of beam-walking test. More recently, Page and collaborators generated a novel mouse, by using a tyrosine hydroxylase (TH) promoter to selectively induce ΔtorsinA expression in dopaminergic neurons (Page *et al.*, 2010). These mice present only a basal hypoactivity and deficit in motor coordination during the beam walking test. Striatal basal DA levels did not show any change, but voltametric measurements after treatment with cocaine demonstrated an imbalanced striatal DA release (Page *et al.*, 2010).

Homozygous KI of the ΔGAG mutation or KO of torsinA are both lethal at birth, whereas heterozygous KI mice showed hyperactivity in the open field test

and an altered learning ability during beam walking (Dang *et al.*, 2005; Goodchild *et al.*, 2005). Mutant mice present also abnormal levels of DA metabolites, and in the pontine nuclei torsinA-ubiquitin positive inclusions have been reported (Dang *et al.*, 2005) (Table III).

The KD mouse model expresses a reduced level of torsinA protein. Their phenotype appears very similar to that of KI heterozygous mice, with altered levels of DA metabolites (Dang *et al.*, 2006).

It is worth noting that some relevant commonalities exist among the different models described. First, in all the models a significant impairment of motor learning is observed. This is of relevance, as it implies a disruption of synaptic plasticity processes. Secondly, most of these mice exhibit an alteration of DA neurotransmission, specifically in the striatal region, a result which is consistent with compelling clinical evidence. However, it appears evident that these mouse models exhibit also considerable differences, at multiple levels. The behavioral, biochemical, molecular characteristics of each model may be attributed either to the distinct types of promoter used or to the differential inherent genetic background.

## III. Spontaneous Mutants

Within the group of vertebrates several spontaneous genetic mutations are related to the appearance of dystonic symptoms (Table IV). Unlike genetic engineering models, the presence of spontaneous mutations provides a useful tool to

Table IV
Spontaneous Mutants.

| Disorder(s) | Model | Gene | Protein | References |
|---|---|---|---|---|
| Generalized Dystonia | $dt^{SZ}$ hamster | Unknown | Unknown | Richter *et al.*, 1991 |
| Generalized Dystonia | *dt* rat | ATCAY | human caytaxin homologous | Lorden *et al.*, 1984 |
| Dystonia musculorum mouse | Dystonia musculorum mouse | BPAG1 | Neural isoform bullous pemphigoid antigen | Brown *et al.*, 1995 |
| SCA6, FHM, EA-2 | Tottering mouse | CANCA1A | α1A calcium channel calcium channel | Fureman *et al.*, 2002 |
| DYT12 | Myshkin (Myk) mouse | ATP1A3 | $Na^+$-$K^+$-ATPase α3 isoform | Clapcote *et al.*, 2009 |
| DYT5 | hph-1 mouse | Unknown | GTP cyclohydrolase type 1 | Hyland *et al.*, 2003 |

the study of dystonia, because spontaneous mutant dystonic animals (SMDA) generally exhibit severe motor disturbances that, at least in part, resemble the clinical and characteristics of human dystonia (Hess and Jinnah, 2005; Neychev *et al.*, 2008). Among SMDA, it is worth mentioning the *dt* rat, which develops a dystonic motor syndrome by postnatal day (PND) 12, involving both limbs and trunk muscles, dies before PND 40. The mutation has been identified in the gene encoding a protein having a high homology with human caytaxin (LeDoux, 2010). This protein plays an important role during development of the cerebellar cortex suggesting its involving in dystonia and ataxia (LeDoux, 2010). Indeed, deficiency of caytaxin disrupts phophatidylinositol signaling pathways, calcium homeostasis, and extracellular matrix interactions (Xiao *et al.*, 2007). Histological examination of the striatum, cerebellum, and deep cerebellar nuclei (DCN) revealed no anomalies and no loss in cell number (Lorden *et al.*, 1984, 1992; LeDoux, 2010; McKeon *et al.*, 1984). However, several neurochemical abnormalities were observed within the cerebellum. First, the GABA concentration is significantly elevated in *dt* rat Purkinje cells, but not in the basal ganglia (Lutes *et al.*, 1992; Oltmans *et al.*, 1984), while cells of the DCN show a substantial reduction in glutamic acid decarboxylase (GAD) activity and GABA receptor density (Beales *et al.*, 1990; Oltmans *et al.*, 1984). Moreover, electrophysiological experiments in *dt* rat revealed the presence of a defect in climbing fiber input to cerebellar Purkinje cells, through a reduced rate of complex spiking and abnormal patterns of simple spike bursting (LeDoux and Lorden, 2002). Surgical treatment as cerebellectomy (CBX) or lesions of the DCN are able to relieve the symptoms in *dt* rats (LeDoux *et al.*, 1993, 1995). Together, these results support a primary role of the cerebellum in motor dysfunction of *dt* rats.

Hamster $dt^{SZ}$ is another model implying spontaneous mutation, which has been extensively characterized (Table IV). Genetic screening has not identified the gene that may cause motor dysfunction although it is transmitted as a single autosomal recessive trait (Richter and Loscher, 1993). The dystonic attacks are induced by stressful episodes, and they can last for hours with a large spectrum of severity (Loscher *et al.*, 1989). Although histological examination of the entire brain appears normal, hamster $dt^{SZ}$ exhibit a reduction in striatal parvalbumin-immunoreactive neuron numbers suggestive of an altered GABAergic transmission (Gernert *et al.*, 2000; Hamann *et al.*, 2007). Conversely, the number of other classes of striatal interneurons is unaffected (Gernert *et al.*, 2000).

The hypothesis that an intrinsic deficit in neuronal function underlies dystonia in hamsters has been confirmed through the application of GABA receptor antagonists that worsen dystonic attacks. Conversely, GABA receptor agonist application reduces motor abnormalities (Fredow and Loscher, 1991; Hamann and Richter, 2002; Sander *et al.*, 2009). Together, electrophysiological experiments and pharmacological data demonstrate an overactivity linked to decreased GABAergic inhibition, resulting in profound effects on the processing of basal

ganglia output (Gernert *et al.*, 1999, 2002; Hamann *et al.*, 2010; Kohling *et al.*, 2004). In addition, the administration of dopamine receptor antagonists prevents dystonic attacks, suggesting a dopaminergic dysfunction, but only during the dystonic attacks, since the interictal levels are normal (Hamann and Richter, 2004; Rehders *et al.*, 2000).

The *tottering* mouse model presents a mutation in the *CACNA1A* gene that codes the pore-forming $\alpha_1$2.1 subunit of voltage-dependent Cav2.1 calcium channels (Ophoff *et al.*, 1996; Zhuchenko *et al.*, 1997). These animals belong to a larger dystonic rodent group showing alteration in calcium channels, termed Cav2.1 calcium channels mouse mutants (CCMM) (Raike *et al.*, 2005). The mutation is recessive, and the motor dysfunction, also associated with mild ataxia, is transient and precipitated by stressors (i.e., caffeine or ethanol administration) (Fureman *et al.*, 2002). Although CCMM do not show any evident neurodegeneration, the total volume of the cerebellum is significantly lower (Isaacs and Abbott, 1995). CCMM animals present a decline of the density of Purkinje cells, with a diminished Cav2.1 activity associated with a decrease in the expression of calcium-binding proteins (Dove *et al.*, 2000; Herrup and Wilczynski, 1982). In CCMM, dystonia appears to be linked to the activation of c-fos expression within the entire olivocerebellar network, but not within the basal ganglia (Campbell and Hess, 1998).

The description of spontaneous mutants highlights the existence of a largely variable spectrum of behavioral, neurochemical, and histological abnormalities. Such heterogeneity appears to reflect the intimate, region-specific, consequences of the mutation.

## IV. Pharmacological and Neural Lesion Models

### A. Nonhuman Primates

Different nonhuman primate (NHP, mostly *Macaca Mulatta, Cebus Apella*) models of dystonia have been developed in the past. These models were obtained by lesioning specific brain regions or by pharmacological manipulations within various basal ganglia areas, and for this reason they are still of great interest since they allowed a fine exploration of the neural pathways underlying dystonia (Table V). NHP models allow investigation of some aspects of dystonia pathophysiology that are inaccessible with the noninvasive techniques used in human patients. Thus, in principle, NHP represent the most reliable animal species to model neurological disorders, such as dystonia. Indeed, primates are typically employed for preclinical testing of novel pharmacological agents, or neuromodulatory devices. However, several factors limit their extensive use. NHP, in fact, breed very slowly and in a few

Table V
PRIMATES, PHARMACOLOGICAL, AND NEURAL LESION MODELS.

| Disorder(s) | Species | Treatment | Symptoms | References |
|---|---|---|---|---|
| Cervical dystonia | *Macaca mulatta* | Electrolytic lesions-medial midbrain tegmentum | Contraversive torticollis | Foltz *et al.*, 1959 |
| Cervical dystonia | *Cercopithecus sabaeus* and *Macaca mulatta* | Lesions of mesencephalic tegmentum | Ipsiversive torticollis | Battista *et al.*, 1976 |
| Cervical dystonia | *Macaca fascicularis* | Microstimulation (muscimol) interstitial nucleus of Cajal | Ipsiversive head rotation | Klier *et al.*, 2002 |
| Cervical dystonia | Monkey | 6-OHDA nigrostriatal lesion | Ipsiversive torticollis that resolved with apomorphine | Sambrook *et al.*, 1979 |
| Cervical dystonia | *Macaca fascicularis* | Bicuculline in globus pallidus pars internalis | Limbs dystonia | Burbaud *et al.*, 1998 |
| Multifocal dystonia | *Macaca fascicularis* | Bicuculline in thalamic relay of pallidal inputs | Tonic hemidystonia | Macia *et al.*, 2002 |
| Occupational dystonia | Monkey | Intensive sensorimotor training | Hand-forearm dystonia | Byl *et al.*, 1996 |

numbers, resulting in a limited available sample number, and their maintenance requires expensive and specific facilities. In addition, primates are potential holders of a number of diseases including retroviral zoonoses, which have had a history of jump host species (Weiss, 1998). Furthermore, ethical issues also contribute to the limited use of these models.

Compelling evidence suggests that dysfunction of the basal ganglia circuit plays a pivotal role in the pathophysiology of dystonia, in agreement with neuroimaging studies (Asanuma *et al.*, 2005; Augood *et al.*, 2004; Carbon and Eidelberg, 2009), as well as with the observation that secondary dystonia occurs in patients with focal lesions of basal ganglia, especially caudate and putamen (Bhatia and Marsden, 1994). Accordingly, experimental evidence collected from NHP models focused primarily on the role of basal ganglia in the pathophysiology of dystonia although other subcortical nuclei have been involved in focal dystonia (Guehl *et al.*, 2009). Regional lesions and pharmacological manipulation of the basal ganglia of NHP have been shown to produce dystonic symptoms (Breakefield *et al.*, 2008; Guehl *et al.*, 2009; Jinnah *et al.*, 2008). The typical phenotype of NHP dystonic model includes torticollis, forced jaw opening and abnormal postures of the limbs, manifestations that closely resemble the clinical syndrome seen in human patients (Guehl *et al.*, 2009; Sassin, 1975).

Acute dystonic reactions are commonly observed in patients undergoing neuroleptic treatment, especially haloperidol (Marsden and Jenner, 1980). In the 1970s, it was shown that such reactions were inducible in primates, producing a clinical syndrome very similar to that reported in human subjects (Bedard *et al.*, 1977). Moreover, as in humans, tardive dystonia can be induced in monkeys by chronic treatment with neuroleptics such as haloperidol (Barany *et al.*, 1983; Kistrup and Gerlach, 1987; Klintenberg *et al.*, 2002). Dystonic manifestations could also be induced by chronic treatment with levodopa in MPTP-treated monkeys (Mitchell *et al.*, 1990). The clinical pharmacology of these dystonic phenomena indicates that they are closely linked to an aberrant striatal dopaminergic signaling (Marsden and Jenner, 1980; Wichmann, 2008). Consistently, as for acute dystonia, drugs that prevent dopamine storage (reserpine) or synthesis (a-methyl-*p*-tyrosine) decrease tardive dyskinesias and dystonia (Chase, 1972).

Other pharmacological models of dystonia in NHP were developed by manipulating the GABAergic system within the basal ganglia. Accordingly, injections of bicuculline, a selective antagonist of $GABA_A$ receptors either in the globus pallidus internalis (GPi) or in the substantia nigra pars reticulata, were able to cause dystonic postures in contralateral limbs or axial symptoms, respectively (Burbaud *et al.*, 1998) (Table V). Conversely, microinjections of muscimol, a GABAergic agonist into the GPi, caused co-contraction of wrist muscles (Mink and Thach, 1991).

Although the role of motor thalamus is still poorly characterized, it certainly represents a crucial relay area for sensorimotor integration. There is robust evidence to suggest that aberrant neuronal activity in thalamic nuclei is related to dystonic movements (Carbon *et al.*, 2009; Lenz and Byl, 1999). Accordingly, abnormalities in the integration between sensory inputs and motor function are considered to play a primary role in the pathogenesis of this disorder (Tinazzi *et al.*, 2009). Pharmacological manipulation of the thalamus lead to distinct phenotypes, according to the specific nucleus involved. In fact, bicuculline injection into the rostral part of the thalamus induced contralateral dystonia with muscle co-contractions, whereas injection in the caudal region caused myoclonic dystonia (Guehl *et al.*, 2000; Macia *et al.*, 2002). These same authors demonstrated that bicuculline was able to increase the discharge frequency of thalamic neurons (Macia *et al.*, 2002).

Taken together these results suggest, as predicted by traditional archetypes of basal ganglia dysfunction, an hyperexcitability of the thalamo-cortical pathway (DeLong, 1990), since focal inactivation of output basal ganglia regions impairs the capability to select between competing motor patterns, thereby interfering with voluntary movements (Mink, 1996).

More recently, models of secondary dystonia were developed in NHP. Primates were chronically treated with 3-nitropropionic acid, a selective mitochondrial complex II toxin, which has been shown to induce motor disturbances in humans (Ludolph *et al.*, 1991). After several weeks, animals developed a dystonic

syndrome, initially confined to lower limbs, but showing a slow and progressive tendency to diffuse to other body parts (Palfi *et al.*, 2000) A plausible interpretation of these effects can reside in the specific capacity of 3-NP to affect energy metabolism of MSNs, ultimately leading to neural dysfunction, and eventually to cell death (Calabresi *et al.*, 2001).

### B. Rodents

Pharmacological models of dystonia have been developed also in wild-type rodents. Both generalized and focal dystonia can be induced through manipulations of different brain regions, such as basal ganglia, cerebellum, and red nucleus (Table VI).

In rats, systemic administration of 3-nitropropionic acid (3-NP) produced generalized motor disturbances consisting of hindlimb clasping and dystonia, truncal dystonia, bradykinesia, and impaired postural control. Histopathologically, there were discrete lesions of the dorsolateral striatum together with a reduction of striatal volume (Fernagut *et al.*, 2002). However, striatal neuronal loss was accompanied also by significant dopaminergic cell damage within the substantia nigra pars compacta, suggesting that a combined dopaminergic denervation was necessary for the dystonic symptoms to occur (Fernagut *et al.*, 2002).

In another study, a single systemic injection of 3-NP in rats caused hindlimb dystonia early after 3-NP injections, and rats performed poorly on balance beam

Table VI
Rodents, Pharmacological, and Neural Lesion Models.

| Disorder(s) | Species | Treatment | Symptoms | References |
|---|---|---|---|---|
| Generalized dystonia | Mouse/rat | Systemic 3-nitropropionic acid | Mild dystonia | Akopian *et al.*, 2008 |
| Generalized dystonia | Mouse/rat | Systemic 3-nitropropionic acid | Dystonia, bradykinesia | Guyot *et al.*, 1997 |
| Generalized dystonia | Mouse | Injection of kainic acid into cerebellar cortex | Severe truncal and appendicular dystonia | Pizoli *et al.*, 2002 |
| Generalized dystonia | Mouse | Systemic administration of L-type calcium channel agonists | Severe truncal and appendicular dystonia | Jinnah *et al.*, 2000 |
| Generalized dystonia | Rat | Injection of sigma receptor ligands into the red nucleus | Cervical and truncal dystonia | Walker *et al.*, 1988 |
| Generalized dystonia | Rabbit | Fetal hypoxia-ischemia | Mixed dystonia-spasticity | Derrick *et al.*, 2004 |

and rotarod motor tests 24 h later (Akopian *et al.*, 2008). Electrophysiological recordings from striatal slices showed an increase in NMDA receptor-dependent LTP at corticostriatal synapses, 24 h after injection (Akopian *et al.*, 2008). These alterations were not due to an increment of NMDA receptor numbers but were D1 DA receptor dependent and were reverted by exogenous addition of dopamine or a D2 DA receptor agonist. Additionally, HPLC and fast-scan cyclic voltammetry revealed a decrease in DA content and release in rats injected 24 h earlier with 3-NP, further supporting a role for dopaminergic innervations of striatal neurons in the pathogenesis of the motor sequelae induced by 3-NP. Furthermore, immunohistochemical analysis showed no evidence of both striatal and nigral cell loss (Akopian *et al.*, 2008).

Another rodent model has been developed by Pizoli and collaborators in 2002, through microinjections of the AMPA-glutamate receptor agonist kainate in the cerebellar vermis. Dystonic movements started from the hindlimbs and spread to the trunk and forelimbs in about 10–20 min after drug injection. The dystonic effects seem causally linked to a cerebellar dysfunction, since kainate microinjection in the basal ganglia did not lead to any apparent motor dysfunction (Pizoli *et al.*, 2002).

In summary, whereas initial clinical studies implicated basal ganglia as the principal source of idiopathic and acquired dystonias, substantial evidence identifies cerebellar dysfunction as another common cause of this disorder. Undoubtedly, the heterogeneity observed in human dystonia is represented in animal models of the disorder. Some studies provide demonstration that dysfunctional output from the basal ganglia can result in dystonia. In these models, abnormal nigrostriatal dopaminergic neurotransmission and impairment of bidirectional synaptic plasticity at corticostriatal synapses predict distorted thalamocortical pathway that may drive the dystonia (Martella *et al.*, 2009; Quartarone and Pisani, 2010; Wichmann, 2008). In contrast, the models of dystonia implicating the cerebellum predict abnormal cerebellar signaling. Considering that both systems serve to modulate motor activity, the notion that simultaneous dysfunction of basal ganglia and cerebellum may be involved in different manifestations of dystonia is not unreasonable.

## V. Conclusions

The past decade has witnessed an important effort in the generation of experimental models for the study of dystonia. The mouse has received considerable attention because of the ease with which genetically defined mutations can be introduced. A major goal in studying these models is to identify the anatomical,

physiological, or biochemical processes involved in the expression of the motor deficits. Extensive characterization of both "genotypic" and "phenotypic" models of dystonia often led to very different results, and as a consequence, generated some skepticism concerning the relevance of studies on rodent to human disease.

For instance, many human studies show that basal ganglia, and more in particular the neostriatum, are central to the origins of dystonia (Bhatia and Marsden, 1994; Breakefield *et al.*, 2008; Perlmutter and Mink, 2004; Quartarone and Pisani, 2010). This region has indeed been implicated in the expression of dystonia in the $dt^{sz}$ hamster as well as in different rodent models of DYT1 dystonia. On the other hand, the cerebellum has been repeatedly implicated in several rodent models, including the *dt* rat and tottering mice.

These diverging experimental data, however, should not be used as evidence to dismiss the models as irrelevant. Conversely, the disputes could be resolved by further investigations of their relevance in human dystonia. In conclusion, the expectation for an animal model to mimic its human counterpart in all respects, from the causative event through the final motor syndrome is increasingly recognized as unrealistic. Indeed, research involving animal models, including invertebrates, suggest that the value of a given model does not rely in how closely it can mimic the human condition. Rather, a useful animal model is judged by how successfully it can be used to explore novel aspects of pathophysiology and therapy.

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# SURGICAL TREATMENT OF DYSTONIA

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Dystonia is a neurological condition characterised by abnormal muscle contractions, often causing repetitive twisting movements or abnormal postures. Varying forms of surgical intervention, for dystonia unresponsive to medical therapy, have evolved over the years and have often been associated with poor outcomes and high morbidity. The advent of stereotactic neurosurgery and the success of deep brain stimulation in treating a number of movement disorders have revolutionized the surgical treatment for dystonia. This chapter reviews the literature concerning the surgical treatment dystonic conditions, from historical origins to the current use of modern functional neurosurgical techniques.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 98
DOI: 10.1016/B978-0-12-381328-2.00021-3




0074-7742/10 $35.00

## I. Background

### A. History of Dystonia

One of the earliest descriptions of dystonia was recorded by Gowers in 1888 (Kandel *et al.*, 1989), whilst Destarac in 1901 used the term "torticollis spasmodique" to describe the twisting neck movements observed in a 17-year girl. Dystonic conditions were identified as distinct from other hyperkinesias by Schwalbe in 1908; however, because of the bizarre nature of the movements, dystonias were originally considered a form of "hysterical neurosis."

The term "dystonia" was first coined by Oppenheim in 1911, who was the first to correctly identify the organic nature of dystonia (Goetz *et al.*, 2001). Focal dystonias however, unlike the generalized condition, have been recognized for centuries. For example, the first recorded case of surgery for spasmodic torticollis was performed in 1641 by German physician Minnius who sectioned the sternocleidomastoid muscle (Kandel *et al.*, 1989).

In 1944 Herz revived the concept of dystonia being an organic disease; however because of failures to identify any specific brain lesions in dystonia, arguments for an organic basis to the condition were not recognized until the 1980s following Marsden's work on cases of hemidystonia (Marsden *et al.*, 1985). In the 1980s and 1990s investigation into generalized dystonia, particularly amongst Jews of Ashkenazi descent, led to the discovery of the DYTI gene mutation at the 9q34 locus. Since then continued research has led to the awareness of a complex array of aetiological causes underlying dystonia.

### B. Prevalence Estimates

Dystonic conditions are not rare. Epidemiological surveys estimate that dystonia is the third most common movement disorder after Parkinson's disease and Essential Tremor (Fahn *et al.*, 1998b), more prevalent than a number of better known neurological conditions such as myotonic dystrophy, myasthenia gravis, and motor neuron disease. In contrast to many other conditions, however, there have been few published epidemiological studies of dystonia. Differences in study design have further confused prevalence estimates, making it difficult to extrapolate data from certain studies to the general population.One of the most comprehensive examinations of prevalence estimates currently available was provided by the Epidemiological Study of Dystonia in Europe (ESDE) collaborative group (ESDE group, 2000). Data pooled from eight countries revealed a prevalence rate of 152 per million for primary dystonia. The highest subcategory prevalence was 117 per million for focal dystonia with segmental dystonia constituting 32 per million and multifocal dystonia 2.4 per million. The commonest form of focal

dystonia was cervical dystonia (57 per million). However, true prevalence is unknown, with many authors suggesting that estimates in published reports are considerably lower than the actual prevalence (Jankovic *et al.*, 2007) due to significant numbers of undiagnosed cases within the community.

## II. Classification

The term dystonia is employed to describe a syndrome characterized by sustained muscle contractions, often causing repetitive twisting movements or abnormal postures. These involuntary movements are caused by co-contractions of agonist and antagonist muscles and are often exacerbated during action but improve with rest, sleep, or sensory tricks (geste antagoniste) such as touching the chin to improve cervical dystonia. Some dystonic movements (e.g., writer's cramp) appear only with specific actions and are referred to as task-specific dystonias. Dystonic conditions are many and varied and can be described either aetiologically or descriptively.

### A. Aetiological Classification

An important aspect of the clinical evaluation of dystonia is the aetiological classification of the condition. This helps in formulating treatment strategies, deciding on the need for genetic counselling and may also aid our understanding of the underlying pathophysiology of the illness. In order to assist the aetiological classification of dystonic conditions, a similar system for the classification of parkinsonism has been adopted by many (Fahn *et al.*, 1998a) to include the subcategories of primary dystonia, dystonia-plus syndromes, secondary dystonia, and heredodegenerative diseases in which dystonia is the prominent feature. The molecular classification of dystonia includes several genetic loci. Currently at least 19 gene loci have been described (Kamm, 2009; Schmidt and Klein, 2010); although it is likely that there are many more dystonia genes that have yet to be discovered.

### B. Descriptive Classification

#### 1. *Age at Onset*

Dystonia can be subdivided into two groups on the basis of age at onset of symptoms. The term early-onset dystonia is often employed when symptoms begin before the age of 26 years, whereas after this age, they are classified as late-onset dystonia. The earlier dystonic symptoms appear, the more likely the condition will

progress to become generalized, whilst in the older onset individuals, it is more likely to remain focal.

2. *Distribution of Affected Body Regions*

When dystonia is classified according to body distribution, it can be described as focal, segmental, multifocal, or generalized. In focal dystonia, a single body region is affected, such as the arm in writer's cramp, the eyes in blepharospasm, the neck in spasmodic torticollis, or the laryngeal muscles in spasmodic dysphonia. In segmental dystonia, two or more contiguous body regions are affected, for example cranial–cervical dystonia or crural dystonia (one leg plus trunk or both legs). If two or more noncontiguous body parts are affected, the disorder is termed multifocal dystonia. Generalized dystonia refers to crural dystonia with at least one other body part involved. When dystonia is confined to one side of the body, it is called hemidystonia.

The site of the first dystonic symptom is also a valuable prognostic indicator. 90% of patients with onset in the lower limbs will develop symptoms in other parts of the body. Their symptoms are also more likely to become generalized (Greene *et al.*, 1995), compared to those who present with cervical dystonia, of which only a minority will have disease spread to other body parts. Hemidystonia is almost always symptomatic regardless of its age of onset (Marsden *et al.*, 1985).

## III. Medical Treatment of Dystonia

One of the first general considerations in approaching the treatment of a patient with dystonia is to differentiate between the primary and secondary dystonias. In a minority of patients with secondary dystonia, for example Wilson's disease, drug-induced dystonia or dopa-responsive dystonia (DRD), benefit can be gained from specific treatments (Jankovic, 1998). For example, all patients with childhood onset dystonias should receive a trial of L-Dopa, as this may bring about dramatic improvement after a short period of time in those with DRD. For other patients, therapy is directed at controlling the symptoms rather than the cause, with different management strategies employed for generalized as opposed to focal conditions. As a general rule, in patients with generalized and multifocal diseases, oral pharmacotherapy constitutes the mainstay of treatment.

Unfortunately the treatment of dystonia with oral agents is often unsatisfactory. In addition to L-Dopa, other established medications include anticholinergics, benzodiazepines, and baclofen. Except in the case of DRD, anticholinergic medications such as trihexyphenidyl are arguably the most effective pharmacotherapy for dystonia (Bressman and Greene, 2000); although effective treatment is sometimes limited by the development of side effects. Although considered less effective than

anticholinergics, baclofen has proved efficacious in children (Greene, 1992; Greene and Fahn, 1992). Benzodiazepines are also often employed, such as clonazepam, and are particularly useful in the treatment of myoclonic dystonia (Das and Choudhary, 2000). The dopamine depleting drug tetrabenazine is considered to be effective in the treatment of some patients with tardive dystonia. Other anti-dopaminergics such as haloperidol, may be effective but may also worsen symptoms or induce tardive dyskinesia and hence are not recommended. Although atypical neuroleptics such as clozapine have been suggested for the treatment of tardive dystonia, their effectiveness in treating other forms of dystonia remains questionable. Several other forms of pharmacological therapy have been reported to be beneficial in individual cases of dystonia; however their role in treating dystonia has yet to be fully established.In contrast, patients with focal dystonia tend to benefit most from treatment with botulinum toxin (BTX) injection. BTX is often the treatment of choice for the majority of focal dystonias, particularly cervical dystonia, and has been the most comprehensively investigated therapy for treatment of this patient group. Injections in the most severely affected muscle groups can also be employed in association with other treatments. BTX produces chemodenervation and hence local muscle paralysis at the neuromuscular junction and also appears to improve reciprocal inhibition by altering sensory inflow through muscle afferent fibres (Priori *et al.*, 1995). There are several serotypes of BTX although currently only types A and B are available for use in clinical practice. The duration of effect for BTX is variable but on average lasts for two to three months. Lack of response to BTX injection may occur if there is long-standing disease with contractures or the development of antibodies. Resistance associated with neutralizing antibodies may occur after repeated injections in 5–10% of cases (Hanna and Jankovic, 1998).

Both BTX A and B have been shown to be efficacious in placebo-controlled trials (Comella *et al.*, 2000; Sycha *et al.*, 2004) with improvements of 80–90% observed in cervical dystonia (Adler, 2000). There are various injection strategies that have been used including the use of EMGs to guide selection of the appropriate muscle groups requiring treatment (Childers, 2003). It is usually a safe treatment that can be performed repeatedly; however side effects including dysphagia, pain at the injection site, dry mouth, flu-like symptoms, and dysphonia have been reported (Dauer *et al.*, 1998). The majority of patients report satisfactory benefit from BTX treatment, however if this and other conservative measures fail, patients should then be considered for surgical intervention.

## IV. Surgical Treatment of Dystonia

More than two centuries after Minnius' operation for torticollis, the Russian surgeon Buyalsky (1850) performed the first spinal accessory nerve section for

spasmodic torticollis, followed by Morgan in 1867 and Collier in 1890 (Kandel *et al.*, 1989). Spinal cord root section to treat spasmodic torticollis was first proposed over a century ago by Keen (Keen, 1891) who suggested unilateral section of the first three anterior cervical roots. Cervical rhizotomy procedures were further refined by surgeons such as Dandy in 1928, who combined cervical root and accessory nerve sectioning. Bertrand provided extensive data based upon experience with a wide range of procedures derived from Keen's original operation (Bertrand *et al.*, 1978; Bertrand and Molina-Negro, 1988). By 1979 variations of this procedure were still considered the operation of choice for cervical dystonia refractory to medical therapy, although long-term follow-up disputed the effectiveness of these techniques (Meares, 1971). The issue of long-term efficacy, together with the high incidence of denervation related complications, has led to the virtual abandonment of these techniques. Microvascular decompression of the accessory nerve, peripheral facial neurectomy, and cervical cord stimulation are further examples of procedures employed that have also fallen out of favor. Although satisfactory results have been reported for extensive muscle resections performed in patients with cervical dystonia, the extreme nature of this surgery has prevented it from being widely used (Chen *et al.*, 1991; Xinkang, 1981).

## V. Deep Brain Stimulation (DBS) for Dystonia

### A. Development

The development of DBS treatment for dystonia dates back to the 1950s, when Hess and Hassler constructed an elaborate animal model to explain both the physiology and pathophysiology of cervical dystonia (Hassler and Dieckmann, 1970). In clinical practice, however, ablative thalamic surgery for dystonia was used almost exclusively for decades (Ondo *et al.*, 2001; Tasker, 1998). Reported results were very variable with about 50% of patients experiencing some degree of benefit. In 1977 when Mundinger reported his early results with thalamic DBS for dystonia, there was little initial interest in his studies (Mundinger, 1977).

The globus pallidus internus (GPi) was suggested to be a suitable target for dystonia based upon the discovery that ablative pallidal surgery brought about marked improvement in the dystonic dyskinesias of Parkinson's disease. Since then, pallidotomy has been reported to be effective in various dystonic disorders (Hutchison *et al.*, 2003; Lozano *et al.*, 1997; Ondo *et al.*, 1998; Yoshor *et al.*, 2001); although it has carried with it the risks of speech and cognitive impairment, as well as a partial recurrence of dystonic symptoms over time. Since its introduction, DBS has replaced ablative surgery for the treatment of dystonia in many neurosurgical centers throughout the world. Initial studies of DBS in dystonia targeted nuclei within the

thalamus with variable outcome. GPi DBS, inspired initially by the success obtained with pallidotomy, is currently the most popular surgical target for dystonia. Thalamic targets can still be considered in patients with secondary dystonias with pathological changes in the pallidum or where GPi stimulation has been unsuccessful. More recently Subthalamic nucleus stimulation has been reported to be effective in focal and segmental dystonia (Kleiner-Fisman *et al.*, 2007).

## B. Preoperative Assessment

Prior to consideration for surgery all patients should be evaluated to assess the severity of dystonia, the level of disability and to screen for secondary causes of dystonia. As well as a thorough neurological assessment, a cognitive and psychiatric assessment is often undertaken to evaluate for cognition and mood disorders that may affect the outcome. A preoperative MRI is required to rule out any structural lesions in the basal ganglia that may interfere with surgical treatment. A preoperative MRI of the cervical spine may also be indicated in order to assess the contribution of degenerative cervical spine disease in those with cervical dystonia. A valid rating scale should also be employed for evaluating the clinical state of a patient with dystonia and should accurately represent the extent of the disease severity as well as the disability caused in relation to activities of daily living.

Burke, Fahn and Marsden produced a rating scale, initially for the therapeutic trial of trihexyphenidyl in the treatment of dystonia. This clinical assessment scale, the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) (Burke *et al.*, 1985), was later developed further for the assessment of primary torsion dystonia. By documenting serial scores, the BFMDRS has been used extensively for following the clinical course and response to therapy of dystonia patients. It is arguably the most widely accepted rating scale for generalized dystonia and hence has improved comparison of dystonia patient data amongst clinicians by providing comparable quantitative information. Although originally designed for assessment of primary generalized dystonias, the scale has also been used to assess secondary and focal dystonias.

There is probably less consensus, compared with other dystonias, as to which outcome measure best monitors response to treatment in patients with cervical dystonia. Several different rating scales have been proposed, including the Columbia rating scale (Greene *et al.*, 1990), Tsui rating scale (Tsui *et al.*, 1986), and Jankovic rating scale (Jankovic, 1982). These scales however have not been validated as extensively as the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS), a scoring system employing a video protocol, which has been used in many clinical reports. Although TWSTRS has its limitations, the presence of a specific videotape protocol helps ensure that patients are assessed in a more consistent manner, hence why TWSTRS has been chosen by most as the rating scale to evaluate patients with cervical dystonia.

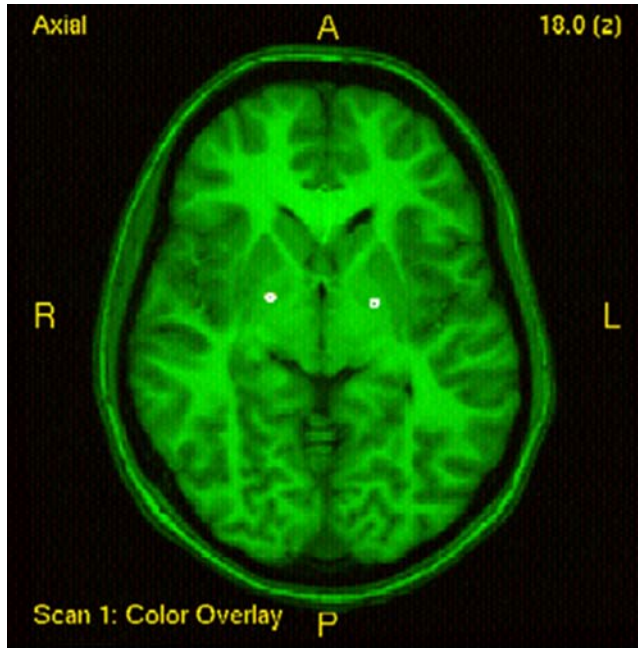


FIG. 1. Fused axial CT and MRI scans following insertion of DBS leads. Stimulator electrodes traversing the GPi are displayed. (For color version of this figure, the reader is referred to the web version of this book.).

### C. SURGICAL CONSIDERATIONS AND TECHNIQUES

The target we employ for dystonia is located in the posteroventral lateral GPi and is the same as that used for pallidal DBS in PD. Our operative technique has been published elsewhere in more detail (Joint *et al.*, 2002). Most patients with dystonic conditions have bilateral stimulation, and the two electrodes are usually implanted in the same surgical session under general anaesthesia (Fig. 1). These electrodes are then connected to a subcutaneous programmable pulse generator usually implanted in the subclavicular tissue. An alternative surgical method employs the use of microelectrode recordings; however this is not routinely applied for stereotactic operations in our centers.

### D. POSTOPERATIVE DBS PROGRAMMING AND PATIENT MANAGEMENT

Improvements following pallidal stimulation may be delayed, and it can take several months before the full benefit is evident (Yianni *et al.*, 2003). The initial stimulation settings are based on a bipolar stimulation mode in accordance with the standard practice at the authors units. Initial stimulator parameters aim for

settings in the region of: 2.0–4.0 V, 130–180 Hz, and 90–240 μs as tolerated by the individual patient with progressive adjustment of electrical parameters at each follow-up visit. Beneficial results have also been achieved with differing parameter settings, particularly with lower frequency stimulation at 60 Hz (Alterman, Miravite *et al.* 2007). This strategy differs slightly to methods employed by other groups who advocate the use of monopolar electrode settings with a maximum of two electrodes (Coubes, Roubertie *et al.* 2000).

During the next few months, the intensity of stimulation is gradually increased, although usually only modest adjustments are required. Side effects of stimulation are reversible upon adjustment of DBS settings. The threshold for undesired effects, such as perioral tightness, dysarthria, dizziness, and paraesthesias, tends to change during progressive adjustments of stimulation amplitude. Weight gain is observed in some patients, but is nonspecific and has also been observed in pallidal surgery for other movement disorders (Krauss, Yianni *et al.* 2004).

DBS has the advantages over lesional surgery of being reversible and adaptable. It avoids concern about the effects of lesioning on the developing brain in children and allows bilateral surgery to be undertaken more safely because of the reduced level of morbidity involved when compared to lesioning. However, DBS is not without its problems, which include hardware failure, high costs, time-consuming follow-up as well as the peri-operative risks of infection and possible intracranial hemorrhage (Joint *et al.*, 2002; Rowe *et al.*, 1999). The overall rate of hardware-related problems reported is very variable ranging from 8–65%, and perhaps reflects differences in surgical technique (Hariz, 2002; Joint *et al.*, 2002; Lyons *et al.*, 2001).

Failure of chronic GPi stimulation may result in a medical emergency such as the rapid and potentially serious reappearance of dystonic symptoms known as "status dystonicus" (Manji *et al.*, 1998; Teive *et al.*, 2005). More often in our groups' experience, hardware failure caused by unilateral lead dysfunction results in a more gradual and progressive recurrence of symptoms, perhaps accounted for by the presence of the retained contralateral stimulation.

So far most studies suggest that GPi DBS does not have a significant adverse effect on cognition or mood although, although to date two suicides have been reported after GPi DBS for dystonia (Foncke *et al.*, 2006).

## VI. DBS for Dystonia—Clinical Overview

Several studies have now shown that DBS, in particularly pallidal DBS, is reasonably safe and efficacious in a variety of dystonic disorders. However, until recently the majority of evidence supporting GPi DBS as an effective treatment

was provided by pilot data comprising a number of case series or case reports. The evidence from these studies has now more recently been strengthened following the results of a number of trials detailing improvements in segmental, generalized and cervical dystonia (Kupsch *et al.*, 2006; Morgan and Sethi, 2008; Mueller *et al.*, 2008). A number of studies with blinded outcome assessments have also been performed which have further added to the evidence base (Diamond *et al.*, 2006; Kiss *et al.*, 2007; Pretto *et al.*, 2008; Vidailhet *et al.*, 2007). Verification of the continued benefit of this treatment has also been provided by recent long-term studies conveying the sustained improvements experienced by patients several years after their initial surgery (Cersosimo *et al.*, 2008; Hung *et al.*, 2007; Isaias *et al.*, 2009; Loher *et al.*, 2008).

Age is generally not a contraindication to dystonia surgery as patients from ages of 8 to 75 years of age have been successfully operated on. Whilst it is still debatable as to whether age of onset of dystonia influences outcome (Vasques *et al.*, 2009a), the overall duration of dystonia has been reported to negatively correlate with poorer post-operative outcomes in a few studies (Isaias *et al.*, 2008). Thus DBS should be considered earlier rather than later in the disease course, in order to prevent secondary fixed deformity that may compromise rehabilitation.

The overall costs of chronic pallidal stimulation are relatively high for patients with dystonia (Yianni *et al.*, 2005). This is partly due to the relatively younger age of patients treated for dystonia compared to those with Parkinson's disease but also due to the comparatively higher energy required for chronic stimulation. Strategies to help address this cost issue have included the development of rechargeable pulse generator batteries.

### A. Cervical Dystonia

Since cervical dystonia is the most frequent dystonic movement disorder DBS might be of considerable interest particularly to those patients who do not respond satisfactorily to conservative interventions. Since the first patients with cervical dystonia have been treated by GPi DBS in the late 1990s, beneficial results have been reported by a number of centers. Bilateral pallidal stimulation produces both symptomatic and functional improvement including marked and sustained relief of pain in patients with cervical dystonia (Krauss *et al.*, 1999). The gradual amelioration of symptoms over months was reflected in our data by improvement of a modified TWSTRS scale on subsequent follow-up examinations, and the mean scores were better at 1 year after surgery than at 3 months postoperatively (Fig. 2). In the patients from our unit, formal follow-up evaluation has demonstrated sustained improvements in the region of 60–65% in overall patient TWSTRS scores. In some patients, relief of pain preceded

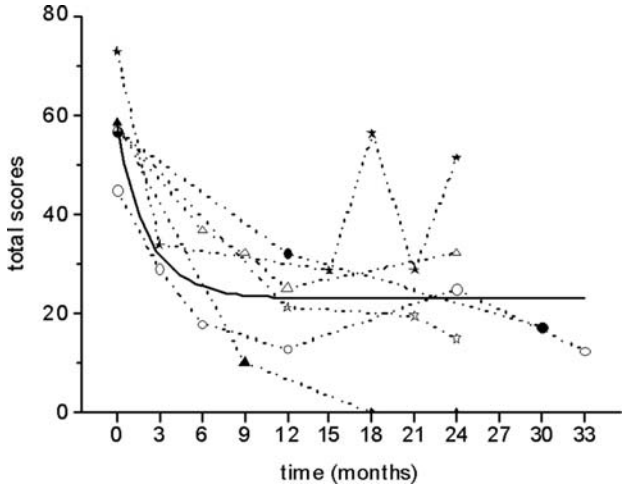


FIG. 2. Effect of GPi DBS on TWSTRS scores of patients with cervical dystonia. Regression curve displays overall change in TWSTRS total scores.

improvements observed in the other aspects of the TWSTRS scale. The literature also reports patients in whom relief of pain was the most prominent feature (Kulisevsky *et al.*, 2000).

A further benefit of GPi DBS in this patient group has been its use as an adjunct in patients with cervical dyskinesias and secondary cervical myelopathy prior to performing spinal surgery or spinal stabilization (Krauss *et al.*, 2002).

### B. Generalized Dystonia

The most beneficial results with pallidal DBS were reported in children with genetic DYT1-positive generalized dystonia. In their first publication on this subject, Coubes and colleagues described a mean improvement of 90% in the BFMDRS at a follow-up of at least 1 year after surgery in seven patients (mean age of 14 years at operation) (Coubes *et al.*, 2000). Improvement was gradual occurring months after implantation of the electrodes. Six children managed to walk without assistance after surgery and became functionally normal. Drugs were reduced in all patients resulting in improvement of alertness. All children returned to school. More recently, beneficial long-term results for a larger number of patients have also been reported by the Montpellier group (Vasques *et al.*, 2009b). Adverse effects have been minimal and several other groups have reported similarly favorable results. Nevertheless, single cases have been reported of patients who did not achieve this expected dramatic postoperative benefit.

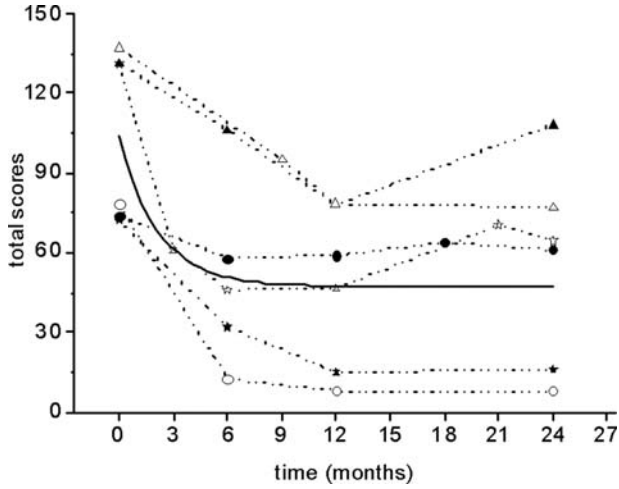


FIG. 3. Regression curve displaying reduction in BFMDRS total scores following pallidal stimulation in patients with generalized dystonia.

It also appears that in adult patients with primary generalized dystonia remarkable benefit is also achieved with bilateral pallidal DBS. Meta-analysed data indicates that a greater than 50% mean improvement in dystonia severity following DBS would be expected in patients with primary dystonias, myoclonus dystonia, certain types of heredo-degenerative dystonia and tardive dystonia (Andrews *et al.*, 2010). In two adult patients with a positive family history of dystonia, BFMDRS motor scores improved by 74% after 2 years and disability scores by 67% (Krauss *et al.*, 2003). In our group 12 patients with generalized dystonia achieved a mean improvement of 48% in the BFMDRS severity scores, and 38% in the disability scores (Yianni *et al.*, 2003) (Fig. 3).

The lesser improvement in our group was most likely the consequence of several factors including greater variability in clinical background, the effect of treatment duration, and the duration of disease onset to treatment, which was on average more than 12 years. It is important to treat generalized dystonia at an early stage (Andrews *et al.*, 2010), particularly before improvement is limited by permanent neurological deficits due to cervical myelopathy, spine deformities or musculoskeletal injury. The response of generalized dystonia to pallidal DBS also relates to the underlying aetiology of the dystonic condition. In general, patients with primary dystonia, particularly DYT1 dystonia appear to respond well (Andrews *et al.*, 2010). Patients with secondary dystonia respond less well (see below), and poorer results are expected in patients with secondary dystonia with structural lesions (Alkhani and Lozano, 2001).

### C. Segmental and Focal Dystonia

Similar to the results for generalized dystonia substantial benefit has been described in patients with primary segmental dystonia. Improvement of cranial dystonias has also been reported in patients with segmental or generalized dystonia (Bereznai *et al.*, 2002; Muta *et al.*, 2001; Vercueil *et al.*, 2001). In a pilot study, bilateral pallidal DBS was performed in a 60-year-old woman with medically-refractory Meige syndrome. At two year follow-up BFMDRS subscores had improved by 92% for eyes, by 75% for mouth, and by 33% for speech and swallowing (Capelle *et al.*, 2003). A further exploration of the use of Pallidal DBS for Meige syndrome and other focal dystonias might be of future interest.

### D. Secondary Dystonia

The outcomes of DBS for the treatment of secondary dystonia appear to be more complex and less predictable than those for primary dystonia (Andrews *et al.*, 2010). Although overall it appears to be less effective in secondary dystonia, pallidal stimulation has been reported as successful in some individual cases (Vercueil *et al.*, 2001). Thalamic DBS has been suggested to be more useful in such cases. Before the routine use of GPi DBS for dystonia, patients with medically-intractable dystonia who were treated in Grenoble underwent thalamic DBS. Approximately half of the patients treated achieved a good functional result, with some improvements also noted in patients with post-traumatic hemidystonia, and postanoxic dystonia with basal ganglia necrosis.

Treatment of choreoathetosis secondary to cerebral palsy is problematic. Bilateral pallidotomies have yielded limited benefit in such patients with objective improvement of up to 42%, but with a high rate of persistent complications (Lin *et al.*, 1999). More promising results have been described in two case reports using GPi DBS (Angelini *et al.*, 2000; Gill *et al.*, 2001). In a 13-year-old boy with cerebral palsy, who presented with a life-threatening "dystonic storm" requiring artificial respiration and continuous sedation, bilateral pallidal DBS resulted in dramatic improvement with restoration of the ability to walk and a less severe degree of residual dystonia seven months after the operation.

Hemidystonia is a typical manifestation of secondary dystonia. In the past, hemidystonia was shown to respond well to thalamotomies, while pallidotomies yielded less consistent improvement (Alkhani and Lozano, 2001; Krauss and Jankovic, 2002). The results with pallidal DBS are rather heterogeneous. While little or no improvement has been reported in some studies (Yianni *et al.*, 2003), unilateral DBS contralateral to the hemidystonia has resulted in improvement of dystonia-associated pain, movements, posture and functional benefit in other patients (Loher *et al.*, 2000).

## VII. Conclusion

GPi DBS is becoming the mainstay of surgical treatment for disabling and medically refractory dystonia. The outcomes following this treatment are often impressive, however cost and availability have been limiting factors for the more widespread use of this technology. Future developments may include the creation of new technologies and the development of carefully conducted studies exploring differing and new surgical targets.

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