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Hoa Huu Phuc Nguyen
M. Angela Cenci *Editors*

Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease

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Editors

Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease

Springer

Editors
Hoa Huu Phuc Nguyen
Institute of Medical Genetics and Applied Genomics
University of Tuebingen
Tübingen, Germany

M. Angela Cenci
Department of Experimental Medical Science
Lund University
Lund, Sweden

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Preface by the Editors

Parkinson's disease (PD) and Huntington's disease (HD) are the paradigms of opposite movement disorders originating in the basal ganglia. On one hand, poverty and slowness of movement (hypokinesia and bradykinesia) are pathognomic to PD and related conditions. On the other hand, excessive and uncontrolled movements are a hallmark of HD. Indeed, the latter condition is the most common genetic cause of involuntary, fleeting and writhing movements (chorea), which is why the disease used to be called 'Huntington's chorea'. Both PD and HD are not only disorders of movement, however. In both conditions, mental processing and mood are affected, and metabolic or autonomic dysfunction cause a range of non-neurological symptoms.

From both etiological and epidemiological standpoints, PD and HD appear as two widely different conditions. PD is the second most common neurodegenerative disease after Alzheimer's and currently affects about 6.3 million people worldwide. It is an age-related disorder lacking an identifiable cause ('idiopathic') in 90 % of the cases. By contrast, HD is a relatively rare familial disease caused by an autosomal dominant mutation in the *HTT* gene. Symptoms of HD commonly become manifest between the ages of 35 and 50 years, but they can begin at virtually any age depending on the CAG repeat length (see below).

The genetic basis of HD was discovered in 1993 by an international collaborative effort spearheaded by the Hereditary Disease Foundation. Since then, several other neurological diseases were found to depend on a similar genetic defect, consisting in the expansion of a CAG (cytosine-adenine-guanine) triplet repeat stretch within the disease-causing gene.

During the past 17 years, it has become increasingly clear that PD has a strong genetic component, too. Since 1997, several genetic mutations have been positively associated with PD in affected families. Beside these monogenic cases, genetic susceptibility has been suggested to underlie the common idiopathic forms of PD. Indeed, recent genome-wide association studies have established that certain common gene variants occur with an increased frequency in people with idiopathic PD. It is however clear that environmental factors, such as exposure to certain toxins, may underlie many cases of idiopathic PD.

Despite their different etiologies, PD and HD have many things in common. Both diseases heavily affect the network functions of the cortico-basal ganglia-thalamo-cortical circuitry. Disturbances in corticostriatal synaptic transmission and atrophic changes of striatal projection neurons are key players in their pathophysiology. And although different events may trigger the primary neurodegenerative process, there are striking commonalities in the pathogenic pathways involved. The commonalities include, misfolding and aggregation of proteins, deficient protein degradation, neuroinflammation, mitochondrial dysfunction, glial pathology, deficits in axonal transport, loss of synapses, glutamate dyshomeostasis, and altered signaling downstream of both dopamine and glutamate receptors. It is therefore not surprising that PD and HD continue to attract the attention of overlapping communities of basic and clinical investigators. In both disease areas, current basic research aims at either determining the mechanisms of neurodegeneration, or improving animal models that will expedite the mechanistic studies. This basic research has already spurred a number of clinical trials of either symptomatic treatments, or approaches to slow the progression of the disease. However, none of the putative disease-modifying approaches thus far tested have yet translated into successful treatments for the human diseases. It is therefore very important to intensify research efforts that can lead to an improved understanding of the pathobiology of PD and HD, revealing new potential therapeutic targets. This kind of research is heavily dependent on the possibility to reproduce key features of PD and HD in simpler models that are accessible to in-depth biological investigation. Animal models are indispensable to unravel non-cell autonomous mechanisms of disease, and the relationship between neurodegeneration and behavioural impairment, or overt neurological deficits can only be addressed in whole-animal models of disease.

We hope that this volume will aid the research on HD and PD by providing an up-to-date coverage of current animal models that can be used to investigate particular pathways, and to link them to both system-level pathophysiology and behavioural abnormalities. No animal models will ever reproduce all the complexity of a human neurological disease, and it is therefore very important for the research community to rely on an articulate repertoire of models tailored to mimic the specific features to be investigated in each study.

The HD part opens with a comprehensive overview of the clinical features of HD by Ghosh and Tabrizi (“Clinical Aspects of Huntington’s Disease”) including available symptomatic treatments and new data from large clinical natural history studies, which have identified potential biomarkers and predictors of disease onset and progression to guide future therapeutic interventions. The next chapter (“The Neuropathology of Huntington’s Disease”, by Waldvogel, Kim, Tippett, Vonsattel, Faull) then provides a detailed description of the current knowledge of neuropathological changes in human HD brains and emphasizes the association of the heterogeneous nature of HD symptomatology with the heterogeneous nature of the neurodegeneration that occurs throughout the different regions of the brain in different HD patients. Chapter by De Souza and Leavitt (“Neurobiology of Huntington’s Disease”) then outlines our current knowledge on the normal

function of huntingtin and the main pathomechanisms by which mutant huntingtin may mediate neurodegeneration in HD.

With the basis set in the three previous chapters, Brooks and Dunnett review in their chapter ("Mouse Models of Huntington's Disease") the similarities and differences in the neurobiology found in the mouse models of HD and the human disease state. Their review also discusses how abnormalities in functional circuitry and neurotransmitter systems impact on the behavioural readouts across the mouse lines and how these may correspond to the deficits observed in patients. While mouse models have provided invaluable information on the pathogenesis and pathophysiology of HD, they might not be the most adequate species for mimicking the human disease. Rats for example have more developed motor learning and motor capabilities. Assays of cognition are more robust in rats than mice, and test more advanced functional aspects. Carreira, Jahanshahi, Zeef, Kocabicak, Vlamings, von Hörsten and Temel provide a comprehensive review ("Transgenic Rat Models of Huntington's Disease") on the transgenic rat models for HD that have been generated so far. However, both rat and mouse model of HD lack the striking neuronal cell loss observed in HD patients. Chapter "Large Animal Models of Huntington's Disease" by Li and Li reviews important findings from these pig, sheep and non-human primate models including a discussion on why neurodegeneration is more readily observed in these models than in rodent models for HD.

Last but not least, Mrzljak and Munoz-Sanjuan provide a thorough and comprehensive review of the current state of therapeutic development for the treatment of HD including ongoing randomized clinical trials in HD as well as the past and present preclinical development of small molecules and molecular therapies at CHDI with an outlook on future directions.

The PD part opens with Chap. "Clinical and Pathological Features of Parkinson's Disease", by Schneider and Obeso, which sets the stage for all the following ones. This chapter reviews the pathological and symptomatic features that need to be considered when creating or validating experimental models of PD. In the next chapter, Johnson and Fox review state-of-the-art symptomatic models of PD having utmost face validity to the human condition ("Symptomatic Models of Parkinson's Disease and L-DOPA-Induced Dyskinesia in Non-human Primates").

Chapter by Cebrian, Loike and Sulzer ("Neuroinflammation in Parkinson's Disease Animal Models: A Cell Stress Response or a Step in Neurodegeneration?") compares and summarizes findings on neuroinflammatory responses in a wide range of toxin-based and genetic models of PD. The following Chap. "Viral Vector-Based Models of Parkinson's Disease" (by Van der Perren, Van den Haute, Baekelandt) provides an overview of current viral vector-based PD models in rodents, both those based on overexpression strategies for autosomal dominant genes (such as α-synuclein and LRRK2) and those based on knockout or knockdown strategies for autosomal recessive genes, such as parkin, DJ-1and PINK1.

The severe cognitive decline occurring in advanced stages of PD is associated with cortical and limbic alpha-synuclein pathology. Hatami and Chesselet have therefore chosen to summarize the cognitive deficits observed in several transgenic mouse lines overexpressing wild-type or mutated alpha-synuclein. The authors also

discuss how these models relate to the disease process in humans ("Transgenic Rodent Models to Study Alpha-Synuclein Pathogenesis, with a Focus on Cognitive Deficits").

In addition to alpha-synuclein, the gene coding for leucine-rich repeat kinase 2 (LRRK-2) is implicated in autosomal dominant forms of PD. A comprehensive summary of the different models employed to understand *LRRK2*-associated PD is provided by Daniel and Moore ("Modeling LRRK2 Pathobiology in Parkinson's Disease: From Yeast to Rodents"). This chapter covers a wide variety of experimental models, including yeast, invertebrates, transgenic and viral-based rodents, and patient-derived induced pluripotent stem cells.

The PD section of the volume closes with Chap. "Models of Multiple System Atrophy" (by Fellner, Wenning, and Stefanova). This chapter gives an overview of the atypical Parkinson's syndrome, Multiple System Atrophy (MSA) and summarizes the currently available MSA animal models and their relevance for preclinical testing of disease-modifying therapies.

As with any book, it is impossible to cover every aspect of the current literature, and some important lines of research may not have been sufficiently covered here due to space restrictions. We are very grateful to all our dedicated colleagues who have made great contributions to this book. We think that all chapters provide an accurate and thorough overview of our current knowledge of the behavioural neurobiology of Huntington's and Parkinson's Disease and hope that the readers will enjoy each chapter as we did, and that this book will be helpful to them in their research efforts to understand and find treatments for these devastating diseases.

Hoa Huu Phuc Nguyen
M. Angela Cenci

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Contributors

Veerle Baekelandt Katholieke Universiteit Leuven, Leuven, Belgium

Simon P. Brooks Brain Repair Group, Division of Neuroscience, Cardiff University School of Bioscience, Cardiff, Wales, UK

João Casaca Carreira Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

Carolina Cebrián Department of Neurology, Columbia University Medical Center, New York, NY, USA

Marie-Francoise Chesselet Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, USA

Guillaume Daniel School of Life Sciences, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Rebecca A.G. De Souza Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada

Stephen B. Dunnett Brain Repair Group, Division of Neuroscience, Cardiff University School of Bioscience, Cardiff, Wales, UK

Richard LM Faull Department of Anatomy with Radiology, Centre for Brain Research, University of Auckland, Auckland, New Zealand

Lisa Fellner Division of Neurobiology, Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

Susan H. Fox Division of Neurology, Toronto Western Hospital, University of Toronto, Toronto, ON, Canada

Rhia Ghosh Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

Asa Hatami Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, USA

Stephan von Hörsten Department of Experimental Therapy, Universitätsklinikum Erlangen, Franz-Penzoldt-Center, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

Ali Jahanshahi Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

Tom M. Johnston Toronto Western Research Institute, University of Toronto, Toronto Western Hospital, Toronto, ON, Canada

Eric H. Kim Centre for Brain Research, Department of Anatomy with Radiology, University of Auckland, Auckland, New Zealand

Ersoy Kocabicak Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; Department of Neurosurgery, Ondokuz Mayis University, Samsun, Turkey

Blair R. Leavitt Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics, Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada

Shihua Li Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Xiao-Jiang Li Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Chaoyang District, Beijing, China

John D. Loike Department of Physiology and Cellular Biophysics, Columbia University Medical Center, New York, NY, USA

Darren J. Moore School of Life Sciences, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; Center for Neurodegenerative Science, Van Andel Institute, Grand Rapids, MI, USA

Ladislav Mrzljak CHDI Management/CHDI Foundation Inc., Los Angeles, CA, USA; CHDI Management/CHDI Foundation Inc., Princeton, NJ, USA

Ignacio Munoz-Sanjuan CHDI Management/CHDI Foundation Inc., Princeton, NJ, USA

Jose A. Obeso Department of Neurology and Neuroscience, Medical School, Clinica Universitaria and CIMA, University of Navarra and CIBERNED, Pamplona, Spain

Susanne A. Schneider University of Kiel, Kiel, Germany

Nadia Stefanova Division of Neurobiology, Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

David Sulzer Department of Neurology, Columbia University Medical Center, New York, NY, USA; Departments of Psychiatry and Pharmacology, Columbia University Medical Center, New York, NY, USA

Sarah J. Tabrizi Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

Yasin Temel Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

Lynette J. Tippett Centre for Brain Research, Department of Psychology, University of Auckland, Auckland, New Zealand

Chris Van den Haute Katholieke Universiteit Leuven, Leuven, Belgium

Anke Van der Perren Katholieke Universiteit Leuven, Leuven, Belgium

Rinske Vlamings Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

Jean-Paul G. Vonsattel Department of Pathology, Presbyterian Hospital, Columbia University, New York, USA

Henry J. Waldvogel Department of Anatomy with Radiology, Centre for Brain Research, University of Auckland, Auckland, New Zealand

Gregor K. Wenning Division of Neurobiology, Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

Dagmar Zeef Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands; European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

Part I
Behavioral Neurobiology of Huntington's Disease

Clinical Aspects of Huntington's Disease

Rhia Ghosh and Sarah J. Tabrizi

Abstract Huntington's disease (HD) is a devastating inherited neurodegenerative condition characterized by progressive motor, cognitive, and psychiatric symptoms. Symptoms progress over 15–20 years, and there are currently no disease-modifying therapies. The causative genetic mutation is an expanded CAG repeat in the *HTT* gene encoding the Huntingtin protein, and is inherited in an autosomal dominant manner. In this chapter we discuss the genetics, clinical presentation, and management of this condition, as well as new data from large-scale clinical research studies on the natural history of HD.

Keywords Huntington's disease · Chorea · Genetics · Symptoms · Management · Natural history

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R. Ghosh · S. J. Tabrizi (✉)
Department of Neurodegenerative Disease, UCL Institute of Neurology, London, WC1N 3BG, UK
e-mail: tabrizipa@ucl.ac.uk

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1 Introduction

Huntington's disease (HD) is a devastating inherited neurodegenerative condition characterized by progressive motor, cognitive, and psychiatric symptoms. It was formally described for the first time in 1872 by George Huntington, at the time a newly qualified doctor in the USA (Huntington 1872). The clinical features that he observed remain true today and sadly the disease is still "*one of the incurables*", although several studies to find disease-modifying treatments are underway.

Since the original description, the name of this condition has been changed from Huntington's chorea to Huntington's disease to acknowledge the multiple non-motor symptoms faced by patients with this disease; these may cause as much if not greater distress to patients and their families, and management must be tailored to meet these needs appropriately (Novak and Tabrizi 2010). In this chapter we detail the genetic origins, clinical presentation, and current management of HD.

2 Epidemiology

The prevalence of HD is variable across the world. Japan, South Africa, and Finland have naturally very low rates of disease. It has previously been thought to affect 4–10 per 100,000 people in the Western hemisphere (Harper 2002); however, recent data from the UK suggest a higher prevalence at 12.3 per 100,000 (Evans et al. 2013; Rawlins 2010). For many reasons, including those dating back to the seventeenth century when witchcraft was thought to be associated with HD, there has been an enormous amount of stigma attached to this condition (Wexler 2010). This has led to patients and families with HD attempting to conceal their situation rather than seek medical help, thus leading to lower estimations of prevalence.

It is thought that HD spread across the globe due to migration from North-West Europe. There are communities in which the prevalence of HD is exceptionally high; one of the most well-known is the population living near the edge of Lake Maracaibo in Venezuela where the prevalence is 700 per 100,000. It was in this group of people that genetic linkage studies ultimately led to the discovery of the

causative gene mutation for HD in 1993 (Gusella et al. 1983; The Huntington's Disease Collaborative Research Group 1993).

3 The Genetic Origins of Huntington's Disease

Huntington's disease is caused by an elongated CAG triplet repeat in exon 1 of the gene encoding Huntingtin protein ("*HTT*" is used to refer to the gene that encodes the protein "HTT"), which lies on the short arm of chromosome 4. The wild-type gene carries fewer than 36 repeats. Patient's with greater than 40 CAG repeats will develop HD at some point in their lives as the mutation is fully penetrant at these repeat lengths. Those patients who have 36–39 CAG repeats display a reduced penetrance—some may develop features of HD at an older age, others may never become symptomatic at all (Snell et al. 1993; Rubinsztein et al. 1996).

HD is inherited in an autosomal dominant fashion—that is to say, if one parent is affected, there is a 50 % chance that each of their children will be affected. Individuals with "intermediate allele" CAG repeat lengths of 27–35 were previously thought to be asymptomatic, though a recent study suggests that there may be a behavioral phenotype in this group of patients (Killoran et al. 2013). Due to the potential expansion of the CAG repeat length with cell division in meiosis, the offspring of patients who have an intermediate allele may inherit greater than 35 repeats causing symptoms to arise sporadically in a family with no apparent history of HD. Other seemingly sporadic cases of HD occur in cases of non-paternity. Sporadic cases can also arise when the affected parent, who passed on the mutation, died from other causes before developing symptoms, or were misdiagnosed as having primary psychiatric illness or dementia (among others). Therefore, it is important to ascertain a full and accurate family history during assessment. Approximately 6–8 % cases of newly diagnosed HD are sporadic cases (Almqvist et al. 2001; Siesling et al. 2000).

The instability of the CAG repeat length during meiosis can lead to longer repeat lengths in successive generations within a family; longer repeat lengths correlate with earlier age of onset of disease. This is a genetic phenomenon known as "anticipation". It is more likely to occur when the mutation is inherited down the paternal line—hypothesized to be due to differences between spermatogenesis and oogenesis (Kremer et al. 1995; Zühlke et al. 1993). 90 % of patients who have Juvenile HD (with an age of onset of <20 years old) have inherited this from their father (Barbeau 1970).

At a population level, roughly 50–70 % of the variability in age of onset has been shown to correlate inversely with the CAG repeat length; other factors accounting for the remaining variability are a subject of ongoing research and likely to be made up of both further genetic and environmental disease modifiers (Wexler et al. 2004). Therefore, it is not possible to predict age of onset of individual patients seen in clinic—an important point to stress if patients wish to know their CAG repeat length. This is especially true for patients whose repeat lengths range from 40 to 50 (the majority), as great variability is seen in this range.

More recently, the "conditional onset probability model" has been developed, which is more accurately able to estimate the percentage chance of disease-free survival over a set number of years (conventionally 5 years). The model is based on data from a large cohort of 3000 patients and takes into account not only the CAG repeat length, but the number of years disease free that a patient has already lived (Langbehn et al. 2004, 2010). However, even this more sophisticated model cannot accurately predict the age of onset for individual patients, and appropriate counseling must be given with regards to the limitations of this information if disclosing CAG repeat lengths to patients. Indeed, many patients may well be experiencing symptoms of HD before their official "onset" of disease—this is explored more fully below.

4 Clinical Presentation

Huntington's disease is characterized by a triad of progressive motor, cognitive and psychiatric symptoms, with slow but relentless deterioration over a period of 15–20 years. Ultimately, the cause of death is most commonly secondary to pneumonia (Lanska et al. 1988). The mean age of symptom onset is at 40 years, but HD has been diagnosed in children as young as 2 years old, and in adults up to the age of 87. If symptom onset occurs at <20 years of age, the condition is referred to as "Juvenile HD" (Kremer 2002).

Making a formal diagnosis of HD currently requires the presence of definitive extrapyramidal motor symptoms (Huntington Study Group 1996), but the reality is that psychiatric and cognitive symptoms often precede this. Subtle motor features that go undetected by the patient or their family may also be present for years before a diagnosis of "manifest" HD is made (Ross and Tabrizi 2011), as shown in Fig. 1. This is known as the "prodromal" phase of HD, and corresponds to neurobiological changes such as loss of corticostriatal connectivity and striatal atrophy (Tabrizi et al. 2012) (see Fig. 2).

Prior to this patients who are known to carry the genetic mutation are said to have "premanifest" disease, during which they have no subjective symptoms or objective signs on examination. More recently, the term "perimanifest" disease has been used by some, to describe the group of patients with prodromal HD who are felt by their clinician to be developing the extrapyramidal signs that will lead to a diagnosis of manifest HD in the near future (Tabrizi et al. 2012).

4.1 Motor Features

Motor features in HD comprise added involuntary movements and also impaired voluntary movements.

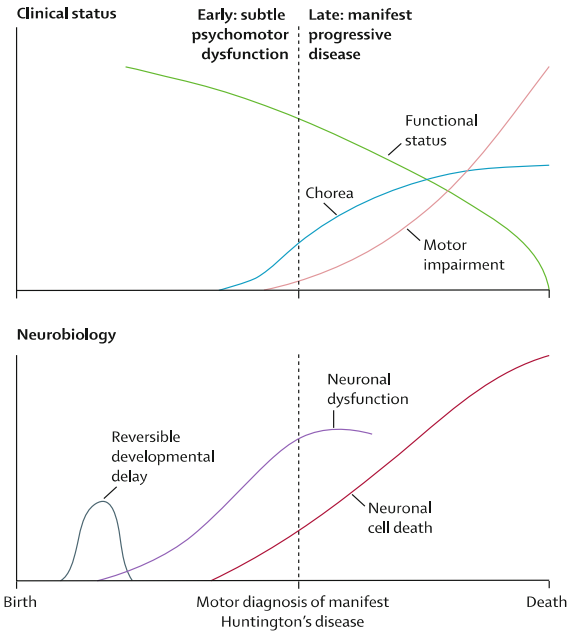

Fig. 1 Progression of Huntington's disease over a patient's lifespan, with corresponding neurobiological changes. Reprinted from Ross and Tabrizi (Ross and Tabrizi 2011) with permission from Elsevier

Initial features include involuntary added movements of the distal extremities and face, which may look like small twitches. Patients may try to incorporate these into their natural voluntary movements. They then spread more proximally, and become larger in amplitude, and the patient suffers from these choreiform movements all the time that they are awake. Chorea is defined as involuntary, excessive movements which are short-lived and can appear to be semi-purposeful. The pattern of the movements also varies, for example, facial muscle involvement can cause eye closure, head turning, and tongue protrusion, whereas involvement of axial muscles causes extension and arching of the back. The degree to which these movements bother the patients themselves is variable, especially in the early stages. However as chorea progresses it can cause problems with writing and eating, and frequently contributes to falling.

Dystonia is also observed—these are slower movements caused by increased muscle tone and sustained muscle contractions which lead to abnormal postures such as tilting or turning of the neck (torticollis) or arching of the back (opisthotonos). As the disease progresses, hyperkinetic movements lessen and instead bradykinesia (slowness of movement), akinesia (delay in initiating movement), and rigidity become more prominent. These later features tend to cause more problems than the more readily recognizable chorea (Novak and Tabrizi 2010).

"Myoclonus" or sudden brief jerking of groups of muscles is sometimes seen in HD, especially in the juvenile form. Likewise, "tics" which are defined as brief, intermittent, stereotyped movements such as blinking, head jerking, nose twitching, or even sniffs, snorts and grunts can develop. These involuntary tics are often

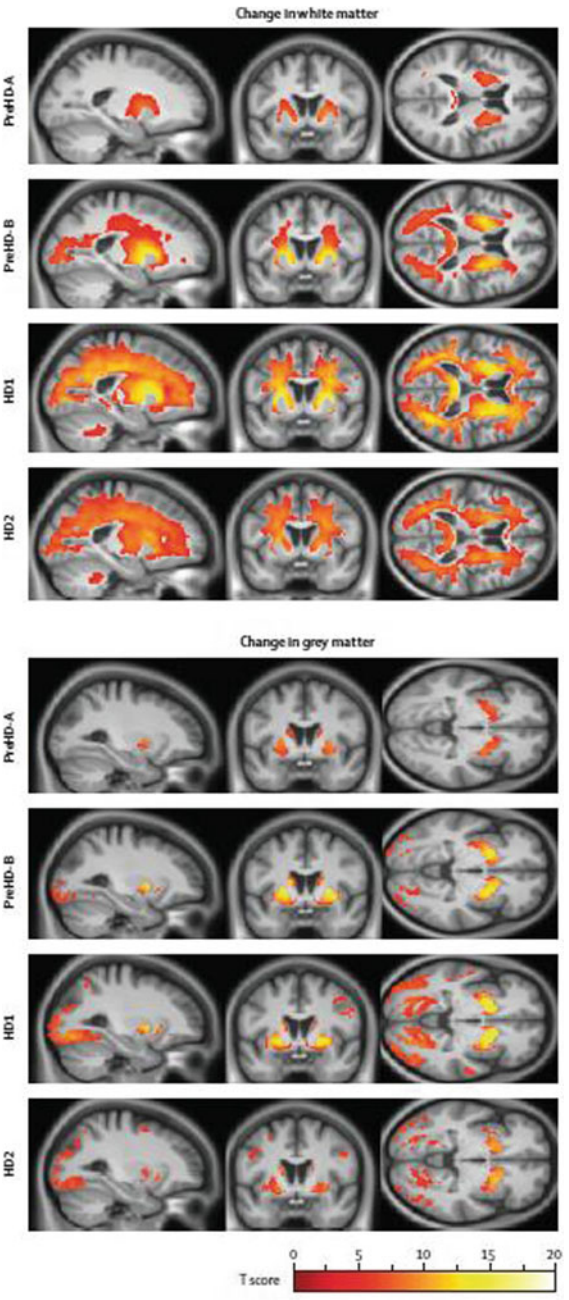

Fig. 2 Parametric maps showing regions with statistically significant atrophy in *grey* and *white* matter at baseline, 12 and 24 months (from *left* to *right* in each frame). PreHD-A and preHD-B are premanifest Huntington's disease gene carriers with estimated time to clinical disease onset greater than and less than 10.8 years, respectively. HD1 and HD2 are patients with early manifest disease who have no functional impairment and mild functional impairment, respectively. The striatum is affected early on, with more widespread atrophy at later stages. Reprinted from Lancet Neurology, Tabrizi et al. 2012 with permission from Elsevier

unnoticed by the patients themselves, but picked up by their carers who can find them irritating.

Gait is affected and may appear ataxic, leading to falls. Loss of postural reflexes also contributes to this. Patients may ultimately require a wheelchair to mobilize safely, and require help with their activities of daily living (ADLs).

Stress, anxiety, and intercurrent infections all lead to temporary deterioration of many of the symptoms mentioned above—a feature that is common to many movement disorders.

4.2 Cognitive Features

Cognitive symptoms may begin during the prodromal phase of HD (Paulsen et al. 2008; Harrington et al. 2012). They are variable in severity, and range from subtle deficits that may go unrecognized by the patient, to prominent impairment. Cognitive problems have been shown to adversely affect daily functional ability independently of motor impairment (Rothlind et al. 1993). Patients however often lack insight into this (and into their disease progression more generally) and for this reason it is important to take a collateral history from relatives and carers. This lack of insight also compounds the cognitive deficits.

The main changes are deficits relating to executive functioning, i.e. high-level cognitive processing which controls other aspects of cognitive functioning. This leads to problems with planning and initiating actions, impaired organizational skills, and inability to adjust to social changes due to the development of concrete thinking. Problems with multitasking are a common early feature in HD and can lead to significant problems in the workplace (Novak and Tabrizi 2010).

Other cognitive problems include general slowing of thought processes, impairments of short-term memory (leading to difficulties in acquiring new skills), and occasionally a decline in visuospatial skills. In particular, patients' perception of their own body in relation to their surrounding environment can be impaired, causing them to bump into objects, trip and fall. Semantic knowledge and language skills are relatively preserved (Craufurd and Snowden 2002).

As cognitive dysfunction progresses, patients can develop a severe frontal and subcortical dementia. However, this does not affect all patients, and must not be misdiagnosed in those patients who have severe motor impairment and are simply unable to express themselves due to a lack of speech.

4.3 Psychiatric Features

Psychiatric symptoms are common in both prodromal and manifest HD, affecting 33–76 % of patients (van Duijn et al. 2007) and occur as part of the underlying disease process rather than simply as a response to the diagnosis. These features often cause more distress and difficulty for patients and their carers than the motor symptoms.

The most common psychiatric condition is depression, followed by anxiety, neither of which relate to the stage of disease (Craufurd and Snowden 2002). Symptoms of depression may be obscured or wrongly attributed to known features of the disease such as weight loss and sleep disturbance but it is important to recognize the condition as treatment is available and effective. A survey of 2835 patients with HD found that 40 % were suffering from depression at the time, and 50 % had sought help for depression in the past (Paulsen et al. 2005).

Suicide is the second most common cause of death in patients with HD (Lanska et al. 1988). In a separate study of 4171 patients, 10 % had made a previous attempt at suicide, and 17.5 % had had suicidal thoughts. Suicidal ideation peaks when premanifest individuals just start to display symptoms, and then again in more advanced disease when loss of independence and functioning occurs (Paulsen et al. 2005). Therefore, it is vital to enquire about suicidal ideation during assessment. Risk factors for suicide in HD include depression and impulsivity (Craufurd and Snowden 2002), but not all patients who make an attempt have depression (Lipe et al. 1993), and some patients feel that suicide is a rational response to their impending loss of independence.

Patients also develop obsessive, compulsive thoughts and behaviors (Paulsen et al. 2001). These include obsessions related to others (e.g. thoughts of infidelity), those related to themselves (e.g. fixations on bladder or bowel function) and ritualistic behaviors (with repetitive routines).

Apathy is common, and characterized by a loss of interest and passive behavior. It can be difficult to distinguish from depression but *is* related to disease stage. Often the difficulty is in initiating activities, and once patients have started on an activity they are able to participate fully.

Patients can sometimes develop irritability and aggression; rarely, this manifests in physical violence. Psychosis (paranoid thoughts and acoustic hallucinations) is a less common feature, seen in later disease stage (Rosenblatt and Leroi 2000). Hyper- and hyposexuality can be a problem in early and late HD, respectively.

4.4 Other Neurological Symptoms

In addition to the triad of movement, cognitive and psychiatric disturbances, patients also exhibit problems with communication, swallowing, and sleep disturbance.

Communication difficulties arise from a combination of dysarthria (caused by incoordination of the orofacial muscles and tongue), cognitive symptoms such as word finding difficulties, and the inability to structure speech appropriately. Patients who have developed the severe rigidity and akinesia seen in advanced HD, may be rendered completely anarthric (mute).

Likewise, swallowing problems arise from a combination of motor (incoordination of oral and pharyngeal muscles) and cognitive impairment. This leads to

increased risk of aspiration. Ultimately, patients may need to establish alternative routes for enteral feeding.

Sleep disturbance is commonly mentioned by patients with HD, and is a cause of significant distress (Videnovic et al. 2009). Insomnia may be secondary to low mood, anxiety, or chorea at night; however, primary sleep disturbance due to presumed dysfunction of circadian rhythms is also recognized. It is important to try and address the cause of insomnia so that treatment may be directed appropriately.

4.5 The Peripheral Phenotype of Huntington's Disease

Huntingtin protein is expressed by all cells in the body, and not only in the nervous system. It is unsurprising therefore, that a range of systemic features are also seen in HD (van der Burg et al. 2009). Blood plasma samples taken from patients with HD show increased levels of IL-6 and IL-8, thus providing evidence for widespread immune activation (Björkqvist et al. 2008). This may contribute to several of the peripheral features described below.

Profound weight loss occurs, greater than that seen in other hyperkinetic disorders. This cannot be explained simply by the difficulties associated with feeding and loss of swallow/manual dexterity, but is thought to be secondary to an underlying catabolic state which occurs as part of the disease process. Often this begins in the prodromal phase of the disease. Patients who have a higher body mass index at disease onset, tend to have a slower rate of progression (Myers et al. 1991).

Skeletal muscle atrophy, despite muscle hyperactivity secondary to chorea, is observed. Cardiac failure is also seen in 30 % of patients (compared to 2 % in age-matched controls) and is a cause of death in HD (Lanska et al. 1988).

Endocrine dysfunction including impaired glucose tolerance and hypothyroidism (reduced T3 levels) is often found in patients. Testicular atrophy, with reduced numbers of germ cells and abnormal seminiferous tubules can be found. Fertility remains unaffected, although men have reduced levels of testosterone. Osteoporosis may also be a part of the peripheral phenotype (van der Burg et al. 2009).

It is important to specifically assess these non-neurological features when reviewing patients in clinic as they can significantly reduce the quality of life (QoL) in HD and may contribute to an early death.

4.6 Juvenile Huntington's Disease

This is characterized by age of onset before the age of 20, and accounts for 6–10 % of all diagnosed HD (Shoulson and Chase 1975). Patients develop rigidity, bradykinesia, and akinesia right from the outset, rather than the chorea that is seen in

Table 1 The Shoulson-Fahn staging system

Stage of disease	Engagement in occupation	Capacity to handle financial affairs	Capacity to manage domestic responsibilities	Capacity to perform activities of daily living	Care can be provided at…
I	Usual level	Full	Full	Full	Home
II	Lower level	Requires slight assistance	Full	Full	Home
III	Marginal	Requires major assistance	Impaired	Mildly impaired	Home
IV	Unable	Unable	Unable	Moderately impaired	Home or extended care facility
V	Unable	Unable	Unable	Severely impaired	Total care facility

adults. Dystonic posturing is also a feature. The first outward signs of disease may manifest as learning difficulties and behavior disturbance whilst at school. Seizures are also present in 30–50 % of patients (Kremer 2002). Generally, these patients have greater than 50 CAG repeats (Andrew et al. 1993) and as mentioned previously, in 90 % of cases paternal inheritance is observed (Barbeau 1970).

The rigid variant of HD is also known as the akinetic-rigid or Westphal variant, and though usually seen in Juvenile HD it also rarely occurs in adults.

5 Disease Progression

From the time of diagnosis symptoms progress over 15–20 years. Assessment scales exist that can be used to quantify disease progression. This is essential for research purposes and also can be useful clinically in guiding interventions such as starting medication or arranging nursing home care. One of the earliest was the Shoulson-Fahn capability scale described in 1979 (Shoulson and Fahn 1979), which divides the disease into five stages and is summarized in Table 1.

Features of this were later incorporated into the Unified Huntington's Disease Rating Scale (UHDRS), which was devised by the Huntington's Study Group (Huntington Study Group 1996). The UHDRS has four components. These are the motor score (comprised of tests for oculomotor function, dysarthria, chorea, dystonia, gait, and postural stability), cognitive tests (assessed by the digit symbols test, Stroop test, and verbal fluency), behavioral/psychiatric assessment (with specific enquiry regarding low mood, guilt, anxiety, suicidal thoughts, aggression, irritable behavior, obsessions, compulsions, delusions, and hallucinations), and a functional capacity assessment [including the total functional capacity (TFC) score]. The motor score of the UHDRS is a commonly used tool in clinic, and is helpful in objectively monitoring motor progression in a clinical setting.

Table 2 The total functional capacity score and its relationship to Shoulson-Fahn stages and clinical descriptors

Descriptor	TFC	Stage
Early	11–13	I
	7–10	II
Moderate or mid	4–6	III
Advanced or late	1–3	IV
	0	V

Giving patients a diagnosis of manifest HD has important implications for their employment, insurance policies and driving, and can have a significant psychological impact on the patient and their family and carers; premature or delayed diagnosis can therefore create problems. Once a diagnosis of manifest HD has been made, the TFC is used to define the disease stage. The TFC scale makes an assessment of the patient's ability to work, complete household finances, chores and ADLs, and what level of care they need and gives an overall combined score from 13 (independent) to 0 (fully dependent). The TFC score relates to the Shoulson-Fahn stage as detailed in Table 2, but when talking to patients and their carers, descriptive terms such as early/moderate or late are often more useful.

A deeper understanding of the natural history of HD may refine the way that we define manifest disease and monitor disease progression (Loy and McCusker 2013; Biglan et al. 2013). This is currently an area of intensive clinical research (see below).

6 Diagnosis and Investigations

The diagnosis is based on the clinical findings in association with a positive family history. Genetic testing allows us to determine the CAG repeat length in individual patients, which confirms the diagnosis. A positive test result has enormous implications not just for the patient, but also for their entire family. Patients should be made aware of this before sending blood for testing and written informed consent for the test must be given.

Neither neuroimaging nor CSF studies are useful in the diagnosis of HD. They are sometimes carried out with patients' express consent as part of ongoing research studies, or on occasion may be useful in excluding other causes of chorea (Wild and Tabrizi 2007).

In those patients who present with chorea in the absence of other cognitive and psychiatric signs, and without a positive family history, the differential diagnosis is wide (Table 3). Chorea secondary to general medical causes such as drugs or systemic illness must be considered and excluded (Roos 2010).

Approximately 1 % of patients who have a history and signs consistent with HD and who are genetically tested return a negative result. These diseases are

Table 3 The differential diagnosis of chorea, including drug induced, systemic and hereditary causes

The differential diagnosis of chorea	
Drug induced	Neuroleptics
	Oral contraceptives
	Anti-epileptics (phenytoin, carbamazepine, valproate, gabapentin)
	Levodopa and dopamine agonists
	Cocaine and amphetamines
Systemic illness	Systemic lupus erythematosus
	Thyrotoxicosis
	Polycythaemia rubra vera
	Hyperglycaemia
	Paraneoplastic
	Infective—HIV and variant Creutzfeldt-Jakob disease
	Post-infective—Sydenham's chorea, herpes simplex encephalitis
	Focal striatal pathology—stroke, space-occupying lesion
Hereditary	Benign hereditary chorea
	Wilson's disease
	Mitochondrial disorders
	Ataxia Telangiectasia
	Lysosomal storage disorders
	Amino acid disorders
	All causes listed in Table 4

known as HD phenocopies (Wild et al. 2008). Possible underlying causes of HD phenocopies are listed in Table 4. For a small proportion of patients, the genetic diagnosis remains as yet unknown. Further research on the phenocopy syndromes may offer insights into the pathogenesis of HD.

7 Management

The management of HD requires a multidisciplinary approach involving neurologists, psychiatrists, general practitioners, physiotherapists, occupational therapists, speech and language therapists, dieticians, and nurse specialists. Referral to a specialist HD clinic is recommended, as these clinics will have developed expertise in managing the condition from many different approaches and will include many of the healthcare professionals mentioned above (Novak and Tabrizi 2010). In addition, HD clinics are able to co-ordinate trials and other research studies, and recruit potential patients when appropriate. As well as being essential in expanding our knowledge of HD and potentially finding better treatments, patients generally find it psychologically beneficial to take part in research. Support from local teams in the community is vital.

Table 4 Diseases that can manifest as Huntington's disease phenocopies with their corresponding genetic cause

Disease	Mutation
Huntington's disease like syndrome (HDL) 1	PRNP—octapeptide insertion in gene encoding prion protein
HDL2	JPH3—triplet repeat expansion in gene encoding junctophilin-3
HDL3	Causative mutation as yet unknown
Spinocerebellar ataxia (SCA) 17 (HDL4)	TBP—triplet repeat expansion in gene encoding TATA-box binding protein
SCA1/2/3	ATXN 1/2/3—triplet repeat expansion in gene encoding Ataxin-1/2/3, respectively
Dentatorubral-pallidoluysian atrophy (DRPLA)	ATN1—triplet repeat expansion in gene encoding atrophin-1
Chorea-acanthocytosis	VPS13A—mutation in gene encoding chorein
McLeod Syndrome	XK—mutation in XK gene on X-chromosome, encoding a supporting protein for Kell antigen on surface of red blood cell
Neuroferritinopathy	FTL—mutation in gene encoding ferritin light chain
Neurodegeneration with brain iron accumulation (NBIA) or Pantothenate-kinase associated Neurodegeneration (PKAN)	PANK2—mutation in gene encoding pantothenate kinase 2
Inherited prion disease	PRNP—mutations in gene encoding prion protein
Friedrich's ataxia	FXN—triplet repeat expansion in gene encoding frataxin

The focus is on symptomatic treatment and on optimizing function, as currently there are no disease-modifying treatments available (Mason and Barker 2009; Bonelli and Hofmann 2007). There is however much research in this field and trials of treatments that potentially alter the course of the disease are underway. This is covered in more detail in a later chapter, and we will focus here on current management.

7.1 Drug Treatments

7.1.1 Movement Disorder

In the early stages, chorea often does not trouble the patient and therefore may not require treatment. As the disease progresses and patients start to have impaired manual dexterity or are at increased risk of falling, drug treatment should be considered. The first-line choice is the dopamine depleting agent tetrabenazine—though it may not completely abolish the excessive movements, randomized

controlled trials have shown that it does reduce choreic movements (Huntington Study Group 2006). Unfortunately, it has been shown to exacerbate or trigger psychiatric symptoms such as depression, which are common in the HD population. Therefore, it is critical to establish a psychiatric history before prescribing this drug.

Tetrabenazine requires a cytochrome P450 2D6 for its metabolism, and this enzyme is inhibited by drugs such as paroxetine and fluoxetine. Thus, the clearance of tetrabenazine would be reduced in this case and serum levels would be raised. Non-inhibitory alternatives such as citalopram or sertraline should be used instead if required (Guay 2010).

For patients with psychiatric comorbidity, or for those who have found tetrabenazine ineffective, the atypical neuroleptic olanzapine may be used. This causes side effects of increased appetite and weight gain, which may actually be useful for HD patients. In addition, it may help with psychiatric symptoms such as agitation, irritability, and anxiety. Caution is needed in patients who suffer from diabetes and regular blood glucose monitoring is required. Furthermore, an ECG should be reviewed prior to starting olanzapine, as on rare occasions the drug causes prolongation of the QT interval. Side effects also include parkinsonism, tardive dyskinesia, sedation, and raised triglycerides.

Other atypical neuroleptics that are prescribed for treatment of chorea are risperidone (which has less effect on appetite) and quetiapine (which has less effect on blood glucose). Both olanzapine and risperidone have been shown to be associated with an increased stroke risk in elderly patients with dementia (Ballard and Howard 2006), so it is important to enquire about other cerebrovascular risk factors prior to starting these drugs.

Older typical neuroleptics such as haloperidol and sulpiride may also be used but carry a greater side effect profile with more parkinsonism, akathisia (an uncomfortable internal sense of restlessness), and tardive dyskinesia. Tardive dyskinesia is a particular concern in HD as it can be difficult to detect in the presence of an existing movement disorder. All neuroleptic drugs carry the risk of neuroleptic malignant syndrome (NMS), but this risk is greater for typical neuroleptics as compared to atypicals. NMS is characterized by the acute onset of delirium, fevers, and rigidity with raised leukocytes and creatine kinase. Though it is rare, it can be life threatening and patients and their carers should be warned about this and advised to seek emergency medical help if any of the above develop whilst taking these drugs. A general principle for all neuroleptic drugs is to start at the lowest dose and titrate up gradually as needed.

When chorea is combined with dystonia, myoclonus, rigidity or spasticity, clonazepam (a benzodiazepine) is useful. Unfortunately it may exacerbate any underlying cognitive impairment, cause sedation and if stopped suddenly withdrawal seizures may occur. Anticonvulsants such as sodium valproate or levetiracetam can be used if myoclonus alone is a significant symptom.

As the disease progresses hyperkinetic movements decline and medications need to be adjusted accordingly, hence the need for regular assessment. Tetrabenazine can be weaned off and stopped, and drugs such as baclofen or tizanidine

may be introduced to address issues of spasticity and rigidity. Injections of botulinum toxin may also be effective in providing symptomatic relief from muscle spasm, and its effects can last for several months before requiring repeat injection. It must however be administered by a specifically trained individual as it can cause unwanted paralysis of muscle groups near to the injection site. For young onset HD a trial of levodopa can be considered if symptoms of rigidity and akinesia are a problem from the outset. A summary of these medications is shown in Table 5.

7.1.2 Psychiatric Symptoms

If features of depression are present then they often respond well to standard antidepressants such as selective serotonin reuptake inhibitors (SSRIs) in the first instance. Citalopram is generally used as first-line treatment; stimulating SSRIs such as fluoxetine can cause hyperstimulation and exacerbate anxiety therefore caution is required. A sedating antidepressant such as mirtazapine, taken at night, is helpful if insomnia is also a problem. Cognitive behavioral therapy (CBT) also plays a role for well-selected patients. Non-stimulating SSRIs may also help with anxiety; occasionally buspirone or benzodiazepines are also used for this.

It is essential to enquire about suicidal thoughts as patients with HD have a high risk of suicide. Psychotic symptoms (though rare) can be addressed with neuroleptic drugs such as olanzapine, which may also help with irritability and aggression. As well as avoiding situations which trigger outbursts, short term use of a benzodiazepine such as clonazepam can be useful for this. Table 6 summarizes some of the drugs that are used in the treatment of psychiatric symptoms.

The choice of drug prescribed should be based on the presence of concurrent symptoms. For example in the case of obsessive–compulsive behaviors or perseveration, neuroleptics may be useful if agitation is present, or alternatively an antidepressant if low mood is a problem. In patients with no or minimal cognitive impairment, CBT can also be useful.

Overall, it is evident that the medications prescribed must be carefully monitored as different symptoms develop and become more or less problematic throughout the disease. The risk of side effects of all of the drugs mentioned must be carefully balanced against the potential benefits they may have.

7.2 Non-Drug Treatments

The management of HD goes far beyond drug prescribing for individual symptoms. Instead, a holistic approach involving many different healthcare professionals is required. Physiotherapists play a vital role in helping to improve gait and balance, and assessment for walking aids or wheelchairs when necessary. In addition, weighted wrist and ankle bands can be provided to dampen distal choreiform movements in the early phase of the disease. Occupational therapists are

Table 5 A summary of pharmacological management of movement disorders in HD

Symptom	Drug class	Drug	Main adverse effects and treatment notes
Chorea	Atypical neuroleptics	Olanzapine	Sedation, parkinsonism, tardive dyskinesias and neuroleptic malignant syndrome but less risk than with older neuroleptics, raised triglycerides, weight gain from increased appetite which may be beneficial in Huntington's disease, caution should be exercised in patients with diabetes and blood glucose should be monitored; may rarely cause prolonged QT interval; useful if the patient also has agitation, irritability, and anxiety
	Atypical neuroleptics	Risperidone	As above but less effect on appetite
	Atypical neuroleptics	Quetiapine	As above but less effect on glucose
	Older neuroleptics	Sulpiride	Agitation, dystonia, akathisia, sedation, hypotension, drymouth, constipation
		Haloperidol	Sedation, more parkinsonism, dystonia, akathisia, hypotension, constipation, dry mouth, weight gain, tardive dyskinesias, higher risk of neuroleptic malignant syndrome than with atypical neuroleptics
	Dopamine depleting agents	Tetrabenazine	Depression and sedation
Myoclonus, chorea, dystonia, rigidity, spasticity	Benzodiazepines	Clonazepam	Sedation, ataxia, apathy, cognitive impairment may be exacerbated, withdrawal seizures
Myoclonus	Anticonvulsant	Sodium valproate	Gastrointestinal disturbance, liver dysfunction, weight gain, blood dyscrasias, hyperammonaemia
		Levetiracetam	Gastrointestinal disturbance, rash, mood changes, myalgia
Rigidity (particularly associated with juvenile Huntington's disease or young adult on set parkinsonian phenotype)	Amino acid precursor of dopamine	Levodopa	Gastrointestinal disturbance, postural hypotension, insomnia, agitation, psychiatric symptoms
Rigidity, spasticity	Skeletal muscle relaxants	Baclofen, tizanidine	Sedation, drowsiness, confusion, gastrointestinal disturbance, hypotension
Bruxism, dystonia	Inhibits acetylcholine release at neuromuscular junction to cause muscle paralysis	Botulinum toxin	May paralyze nearby muscles

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Table 6 A summary of pharmacological management of psychiatric features in HD

Symptom	Drug class	Drug	Main adverse effects and treatment notes
Psychosis	Atypical Neuroleptics	Olanzapine, risperidone, quetiapine	See Table 1. Use carefully in elderly people because of the increased risk of stroke with olanzapine and risperidone in this population
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, gastrointestinal disturbance, tremor, blurred vision
Depression, anxiety, obsessive–compulsive, symptoms, irritability, aggression	Selective serotonin reuptake inhibitors (SSRI)	Citalopram	Gastrointestinal disturbance, hypersensitivity reactions, drowsiness, syndrome of inappropriate antidiuretic hormone secretion (SIADH), postural hypotension
		Fluoxetine	As for citalopram, but also sleep disturbances
		Paroxetine	As for other SSRIs, but also raised cholesterol
		Sertraline	As for other SSRIs
	Presynaptic α_2 adrenoceptor antagonist (increases central noradrenaline and serotonin activity)	Mirtazapine	Weight gain, oedema, sedation, headache, dizziness, tremor; useful when insomnia is a problem because it has sedative properties
	Serotonin and noradrenaline reuptake inhibitor	Venlafaxine	Hypertension, gastrointestinal disturbance, hypersensitivity reactions, drowsiness, agitation, SlADH, palpitations

(continued)

Table 6 (continued)

Symptom	Drug class	Drug	Main adverse effects and treatment notes
Irritability, aggression	Neuroleptics	Olanzapine, risperidone, quetiapine	See above
Altered sleep-wake cycle	Hypnotics	Zopiclone, zolpidem	Drowsiness, confusion, memory disturbance, gastrointestinal disturbance
Mood stabilizers	Anticonvulsants	Sodium valproate	See above
		Lamotrigine	Hypersensitivity reactions, blood dyscrasias, dizziness, gastrointestinal disturbance, depression
		Carbamazepine	Hypersensitivity reactions, drowsiness, blood dyscrasias, hepatitis, hyponatremia, dizziness, gastrointestinal disturbance

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able to carry out home assessments and make relevant adjustments such as grab rails and bathroom adaptations in order to optimize functioning and prolong the length of time that patients can manage at home.

Speech and language therapists are invaluable in offering techniques to help patients communicate verbally for as long as possible. When this is no longer possible, they can provide communication aids in the form of simple charts, or more complex electronic systems that can be used with carers and family. They are also able to assess patient swallowing and recommend the safest consistency of food at each stage of disease. Ultimately, some patients are not able to safely swallow anything orally and may require enteral nutrition through a gastrostomy tube in order to maintain adequate body weight and nutrition, although not all patients choose to proceed with this. It is useful to discuss feeding issues with patients before they reach advanced stages of disease. If gastrostomy feeding is desired, the tube can be inserted as a percutaneous endoscopic gastrostomy (PEG) or as a radiologically inserted gastrostomy (RIG), if an endoscopy is not tolerated.

Involvement of dieticians is required to establish enteral feeding regimes, and prior to this, to advise on dietary adjustments to counteract the catabolic weight loss caused by HD. The role of the social worker is important to advise on and arrange home care, and later on, residential and nursing home care.

Psychologists may be very helpful in providing CBT to help with symptoms of depression and obsessive behaviors, as well as advising on adjustment strategies to

cope with the cognitive and emotional impact of the disease. For example, establishing routine and having set goals to work toward can help to overcome the apathy that affects patients with HD. Local community mental health teams can be very helpful in supporting patients' psychiatric and psychological needs in the community.

There are also many country-specific HD support groups that offer help and advice to patients and also their carers. The European Huntington's Disease Network (EHDN) (www.euro-hd.net) has compiled a map of the HD associations/support groups in various countries across the continent. It also co-ordinates REGISTRY, a cross-sectional observational study of HD (see below). In the USA the HD Association of America (www.hdsa.org) fills a similar role.

7.3 End of Life Care

Though discussions around end of life care can be difficult, it is important to address this issue relatively early on in the overall management of HD, in particular whilst patients still have the capacity to make their own decisions regarding their healthcare (Novak and Tabrizi 2010). In advanced stages of the disease, patients may lose the capacity to make these decisions as cognitive impairment and psychiatric symptoms progress. Therefore, while patients are relatively well their wishes and instructions on how far to take medical interventions in the event of deterioration should be recorded. For example, patients might be happy to receive oral antibiotics at home in the case of a chest infection, but not intravenous antibiotics in hospital.

The legal system varies between different countries, but in general there are two main ways by which patients with HD can ensure that their wishes regarding end of life care are carried out. These take the form of advanced directives, which are legal documents outlining the care or treatment they would or would not want in the event of future deterioration; alternatively patients may nominate an individual as a Power of Attorney who would make decisions on their behalf if they lost capacity in the future. Local support groups and HD clinics have more specific expertise in addressing these issues, and would be best placed to advise on accessing legal assistance if needed. It can bring patients comfort and security, knowing that their wishes would be carried out even if they lost their capacity to make decisions in the future.

As HD progresses, home care becomes less feasible and residential or nursing home care may be required. In the terminal stages patients may prefer to be kept comfortable at home, or in a hospice, rather than dying in hospital and this should be facilitated where possible. This decision could form a part of an advanced directive, as mentioned above. Community palliative care teams, district nurses, and general practitioners can sometimes help to provide symptomatic relief (for example in the form of syringe drivers or subcutaneous injections) at home in order to avoid hospital admission toward the end of life, if this is desired by the patient.

7.4 Genetic Testing

Genetic tests that measure the CAG repeat length in the Huntingtin gene are now readily available and a simple blood test is all that is required.

7.4.1 Diagnostic Testing

Diagnostic testing refers to tests that are carried out in patients who are displaying symptoms of HD. Most commonly they are requested by neurologists to confirm a suspected diagnosis. Before sending the test, patients must be given as much information as possible about the implications of a positive test result, both for themselves and for their family. They should consider when or if they plan to tell their family, and which family members they would tell. If they do return a positive test result, then each of their children also has a 50 % chance of carrying the gene mutation that will lead to HD. Their siblings also would have a 50 % chance of carrying the mutation and might already be developing symptoms of HD. Patients must give written informed consent for this test. If they lack capacity to do so than an authorized representative may do so on their behalf.

7.4.2 Predictive Testing

Predictive testing refers to tests that are carried out in asymptomatic individuals who are at risk of HD due to a positive family history. A positive test result will inform them that they will develop HD at some point in the future, but cannot tell them exactly when this will be. Although statistically there is a strong correlation between CAG repeat length and age of onset (Langbehn et al. 2004), on an individual level it is not possible to make any predictions.

People at risk of HD (i.e., relatives of the affected patient) may want this test as they cannot cope with the uncertainty of the potential diagnosis, or in order to actively plan for the future—particularly in terms of work and reproductive options (Goizet et al. 2002; van der Steenstraten et al. 1994). There are internationally agreed guidelines for predictive testing in HD (Craufurd and Tyler 1992; Went 1990; International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea 1994); tests must be performed at specialist genetic centers, with at least one session of pretest counseling, followed by a period of reflection and then a second counseling session. Post-test counseling should then be available. Again, written consent is required from the patient and as always, strict confidentiality is observed; even the patient's GP is not informed without prior consent.

Children cannot undergo predictive testing as they would lack the adult understanding required to give informed consent. Parents cannot consent on their behalf because the children have the right *not* to know once they become adults

themselves. Furthermore, for a child to grow up knowing that they would develop a devastating neurodegenerative condition could be severely psychologically damaging.

Approximately 5–20 % of patients who know that they are at risk of HD take up genetic testing (Harper et al. 2000). Both positive and negative test results can lead to emotional trauma. In the case of a negative test result, individuals may feel enormous guilt over escaping a disease that has affected others in their family. In the case of a positive result, patients are often in a situation where they have witnessed firsthand the full devastating impact of the disease on their parent and know that they face a similar future themselves.

7.4.3 Reproductive Options

Patients who carry the HD gene mutation face a difficult decision when considering having their own children. Some may choose not to as they consider the risk of passing on the mutation too high at 50 %. This may be compounded by the concern that their own symptoms will develop while their children are young and still requiring care themselves. Others however are prepared to take this risk and have children without seeking further interventions.

If patients do decide to have their own biological children, then prenatal testing is an option. If the parents are certain that they would terminate a pregnancy in which the fetus was carrying the HD mutation, then chorionic villous sampling can be performed at 11–13 weeks gestation (in itself this carries a small risk of inducing a miscarriage) to test for the HD gene mutation. Problems arise if following a positive test result the parents decide to continue the pregnancy, as that child would have a known HD mutation status as a result of a genetic test to which he or she never consented.

In many countries, preimplantation genetic diagnosis (PGD) is a newer option for HD patients. A number of embryos are created using IVF techniques, and tested for the HD gene mutation. The embryos negative for the mutation are then implanted into the womb. Unfortunately, the success rate of a viable pregnancy is around 20 % and patients may require multiple cycles before producing a healthy baby. This is costly and emotionally traumatic, but does ensure that their child is born free of the HD mutation (<1 % chance). Countries where this is permitted include the USA and most Western European countries—the procedure was legalized in Germany in 2011, but is still banned in Switzerland and Austria. Where it is available, it generally remains tightly regulated.

Occasionally patients who are at risk of HD (having an affected parent) may not wish to undergo predictive testing themselves, but want to ensure that their children do not carry the gene mutation. Again, in some countries, linkage analysis on preimplantation embryos can be performed to identify those of <1 % risk or 50 % risk, by testing for genetic markers linked to the *HTT* genes of the affected grandparent. The embryos of <1 % risk would then be selected for implantation.

8 New Data on Mapping Huntington's Disease from Clinical Natural History Studies

Like the global collaborative efforts that lead to the identification of the HD gene in 1993, there have been ongoing multicenter initiatives to study the clinical progression of HD from premanifest through to prodromal, early and advanced HD. This is important not only in helping to define the natural history of the disease, but also offers insights into the underlying pathobiology of HD.

REGISTRY, the largest of these studies to date, is an observational study run by the EHDN (Orth et al. 2011; Orth et al. 2010). Since its beginning in 2004, over 10,000 patients from 16 countries have consented to clinical assessment and thousands have donated biosamples for genetic testing and lymphoblastoid cell line creation, creating an essential database of patients from which research studies and trials can be conducted.

A number of research groups have shown that patients have minor motor signs (Penney et al. 1990; Siemers et al. 1996), cognitive (Kirkwood et al. 1999; Paulsen et al. 2001) and psychiatric symptoms (Snowden et al. 2002) for years prior to a diagnosis of HD, features that make up the so-called "prodromal HD". Several volumetric MRI studies had shown that striatal volume loss was evident up to 10 years prior to disease onset (Aylward et al. 2004). Though all these studies had relatively small sample sizes, they gave us crucial pointers toward the natural history of HD and led to the development of large, multisite, longitudinal studies that underpin our understanding of HD today.

The Neurobiological Predictors of Huntington's Disease "PREDICT-HD" is a longitudinal study following a cohort of over 800 patients from 32 sites with premanifest disease since 2001 (Paulsen et al. 2008). Data is collected at annual visits, including neurological examination (UHDRS motor score, speeded tapping test, self-paced tapping), multiple tests of cognitive function, psychiatric and psychological questionnaires, biannual MRI scans, and blood tests for CAG repeat lengths. Thus far this study has confirmed that motor examination scores, odor recognition, striatal volumes, and cognitive performance is unaffected at 15–20 years from estimated time of diagnosis, but then after a short transition period, a linear decline until disease onset is seen (see Fig. 3).

In terms of cognitive dysfunction, the prominently affected domains in premanifest patients were speed/inhibition, verbal learning/memory, attention-information integration, sensory-perceptual processing, and motor planning/speed; of these, only the latter two were predictive of motor onset (Harrington et al. 2012).

8.1 Biomarkers, and Predictors of Disease Onset and Progression

There is an urgent clinical need to identify biomarkers that will accurately mirror disease progression—this is an absolute necessity when it comes to testing disease-

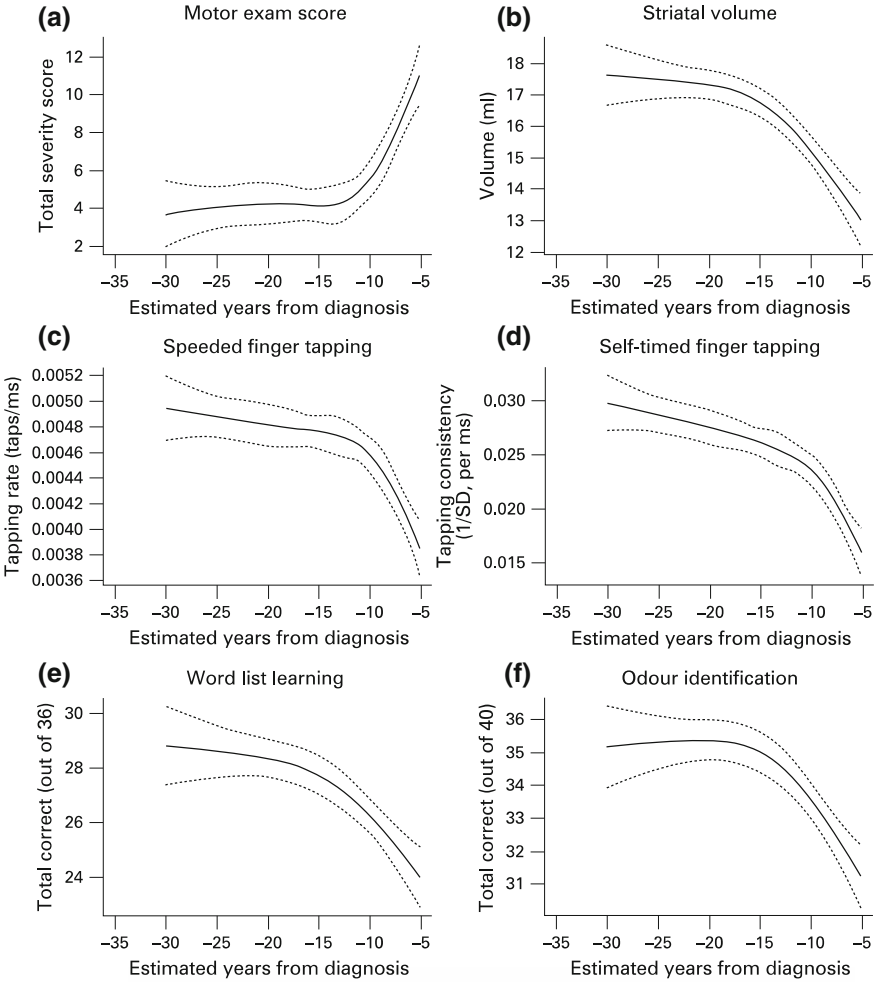

Fig. 3 Relationship between estimated years to diagnosis of Huntington's disease and **a** Motor exam score, **b** Striatal volume, **c** Speeded finger tapping, **d** Self-timed finger tapping, **e** Word list learning, **f** Odor identification. *Solid line* plots the predicted response; the *dotted lines* are 95 % confidence limits for the estimated mean response. Reprinted from Journal of Neurology and Neuropsychiatry, Paulsen et al 2008 with permission from BMJ Publishing Group Ltd

modifying drugs in randomized controlled trials. In addition, factors underlying variations in age of disease onset (other than CAG repeat length) and rates of disease progression, which would potentially help to guide the timing of future therapeutic interventions, are unknown.

To help fill these gaps in knowledge, the TRACK-HD study (Tabrizi et al. 2009, 2011, 2012, 2013) was set up in 2007 to evaluate and deep-phenotype changes over time in 360 participants with premanifest and early HD along with

matched control individuals, with respect to performance on a battery of quantitative motor tests, clinical, cognitive, and neuropsychiatric assessments, as well as a range of advanced neuroimaging techniques with 3T MRI and MRS. Quantitative motor tests included measurements of saccadic eye movements, isometric force on tongue protrusion, self-paced tapping, and stride length variability (Tabrizi et al. 2009, 2011, 2012, 2013). Cognitive tests comprised a battery of 10 tests, including symbol digit modality test, Stroop word reading, circle tracing, recognition of negative facial emotions, a visual working memory task (spot the change), and a smell test (University of Pennsylvania Smell Identification Test). Neuropsychiatric assessment was in the form of a brief structured interview, which was a shortened form of the problem behavior assessment (PBA-s). Functional and QoL assessments included clinician assessments as well as participant self-reporting. Collected over 3 years, this data creates an unprecedentedly detailed map of the natural history of HD, with a large biosample collection of plasma, RNA, DNA, and buffy coat cells.

The data from 36 month follow up of this cohort [by 36 months there were 298 participants: 97 controls, 58 with premanifest HD at least 10.8 years from predicted disease onset (PreHD-A), 46 with premanifest HD less 10.8 years from predicted disease onset (PreHD-B), 66 with early HD and a TFC of 11–13 (HD1), and 31 with early HD and TFC of 7–10 (HD2)] has recently been published (Tabrizi et al. 2013). It confirms robust longitudinal changes in imaging, quantitative motor, and cognitive measures in the PreHD-B and early HD groups. Imaging measures include whole brain, caudate, putamen, white matter, and grey matter volumes. These measures decreased in all 4 patient groups mentioned, with the exception of grey matter volume, which did not decrease significantly in the PreHD-A group (see Fig. 4). These measures could therefore be used to monitor progression in clinical trials on premanifest and early HD patients over 12, 24, and 36 months (Tabrizi et al. 2012, 2013).

Interestingly, premanifest individuals who were greater than 10.8 years from disease onset at enrollment (PreHD-A) did not show significant functional decline over 36 months, although MRI confirmed significant striatal and white matter loss. This suggests that these patients may somehow be compensating for their neuronal loss through synaptic plasticity or enhancement of other neuronal pathways; elucidating these mechanisms is the basis of the ongoing TRACK-ON study.

A particular strength of the TRACK-HD study design is that predictors of disease onset and disease progression were assessed after adjusting for age and CAG repeat length. In terms of disease onset, baseline caudate, putamen, and grey matter volumes (but not white matter volumes) had strong predictive value for future clinical diagnosis in preHD, which in addition to CAG repeat length and age could refine prognostic information for premanifest patients. In particular increasing grey matter atrophy, especially in extrastriatal cortical regions, correlated with a crucial event heralding clinical onset. Baseline total motor score, as well as performance on tasks that assess psychomotor speed and attention, visuospatial working memory and executive function, were also predictors of subsequent clinical conversion to HD.

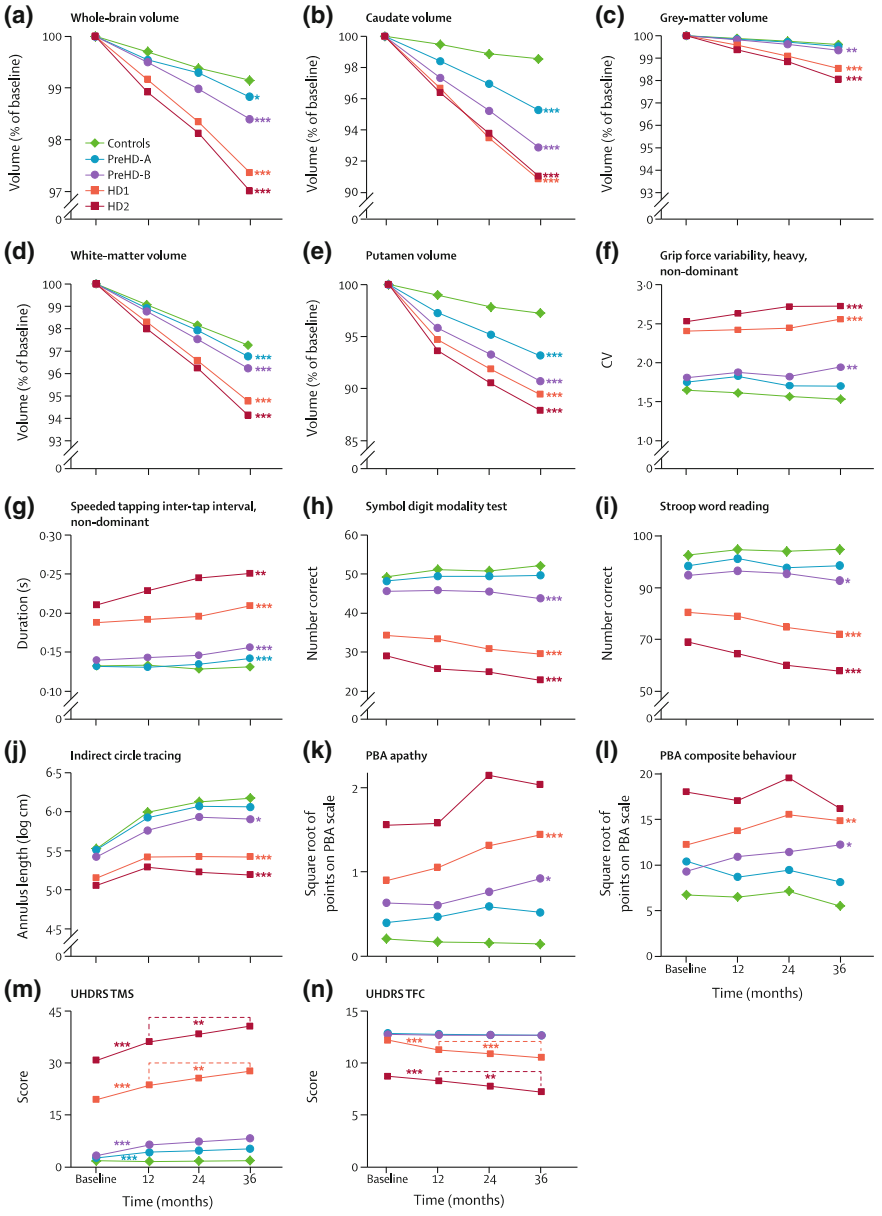

Fig. 4 Changes across a variety of measures from baseline to 36 months in the 4 groups of TRACK-HD. Reprinted from Lancet Neurology, Tabrizi et al 2013, with permission from Elsevier

Rate of disease progression in the early HD groups, as reflected by declining (TFC) scores, were predicted by baseline whole brain, caudate, and grey matter

volumes. Mirrored by the prognostic importance of grey matter changes in the premanifest group, it is again grey matter rather than rather than white matter that is predictive of disease progression in early HD. The non-imaging measures that were predictive of disease onset in the preHD groups were also predictors of progression in early HD, but in addition, the chorea orientation index (a quantitative test of choreiform movement) and baseline apathy scores predicted disease progression.

Longitudinal measures that *tracked* clinical decline over 36 months in the preHD group included higher brain atrophy rates on imaging [both regional (caudate) and global (whole brain, grey matter, and white matter) changes]; impaired performance on the speeded tapping task (one of the few measures that showed significant change even in the preHD-A group); and deterioration in emotion recognition. This latter may explain why lack of empathy is a common complaint from family and friends of patients with preHD.

In the early HD group, clinical decline over 36 months correlated with caudate, whole brain and grey matter atrophy, performance on Stroop test and indirect circle tracing. Emotion recognition also tracked clinical decline, but not at a statistically significant level. In all groups, effect sizes were assessed after adjusting for CAG repeat length and age.

This study also examined the 36 month variances in longitudinal changes, which could be attributed to age and CAG repeat length. In the preHD group this included all imaging changes except grey matter atrophy, thus lending support to the hypothesis that grey matter loss is an independent key marker of disease onset. However, in the early HD group, few relations between CAG repeat length and disease progression were significant (the exception being caudate atrophy).

Thus TRACK-HD has identified imaging and functional measures that could be used to monitor response in multisite clinical trials in the future. Identification of predictors of disease onset and progression will allow appropriate selection and stratification of patient groups in clinical trials, and also furthers our understanding of the natural history of HD.

9 Conclusion

Huntington's disease is a complex neurological disease with varied clinical presentations between patients. The genetic basis for the disease necessarily means that as well as treating individual patients, their entire family needs to be considered and genetic counseling is crucial. Many pharmacological and non-pharmacological treatments are available, though none with any disease-modifying effect as yet. Therefore, management is best achieved through specialist HD clinics that can offer a multidisciplinary approach to the condition.

There is also an intensive international research interest in this condition that aims to better understand the natural history and pathophysiology of this condition, and of course to ultimately discover disease-modifying treatments for this devastating disease.

References

Almqvist EW, Elterman DS, MacLeod PM et al (2001) High incidence rate and absent family histories in one quarter of patients newly diagnosed with Huntington disease in British Columbia. Clin Genet 60(3):198–205

Andrew SE, Goldberg YP, Kremer B et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4(4):398–403

Aylward EH, Sparks BF, Field KM et al (2004) Onset and rate of striatal atrophy in preclinical Huntington disease. Neurology 63(1):66–72

Ballard C, Howard R (2006) Neuroleptic drugs in dementia: benefits and harm. Nat Rev Neurosci 7(6):492–500

Barbeau A (1970) Parental ascent in the juvenile form of Huntington's chorea. Lancet 2(7679):937

Biglan KM, Zhang Y, Long JD et al (2013) Refining the diagnosis of Huntington disease: the PREDICT-HD study. Front Aging Neurosci. doi:10.3389/fnagi.2013.00012

Björkqvist M, Wild EJ, Thiele J et al (2008) A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med 205(8):1869–1877

Bonelli RM, Hofmann P (2007) A systematic review of the treatment studies in Huntington's disease since 1990. Expert Opin Pharmacother 8(2):141–153

Craufurd D, Snowden J (2002) Neuropsychological and neuropsychiatric aspects of Huntington's disease. In: Bates GP, Harper P, Jones L (eds) Huntington's disease, 3rd edn. Oxford University Press, Oxford, pp 62–94

Craufurd D, Tyler A (1992) Predictive testing for Huntington's disease: protocol of the UK Huntington's Prediction Consortium. J Med Genet 29(12):915–918

Evans SJ, Douglas I, Rawlins MD et al (2013) Prevalence of adult Huntington's disease in the UK based on diagnoses recorded in general practice records. J Neurol Neurosurg Psychiatry. doi:10.1136/jnnp-2012-304636

Goizet C, Lesca G, Dürr A et al (2002) Presymptomatic testing in Huntington's disease and autosomal dominant cerebellar ataxias. Neurology 59(9):1330–1336

Guay DR (2010) Tetrabenazine, a monoamine-depleting drug used in the treatment of hyperkinetic movement disorders. Am J Geriatr Pharmacother 8(4):331–373

Gusella JF, Wexler NS, Conneally PM et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306(5940):234–238

Harper P (2002) The epidemiology of Huntington's disease. In: Bates GP, Harper P, Jones L (eds) Huntington's disease, 3rd edn. Oxford University Press, Oxford, pp 3–27

Harper PS, Lim C, Craufurd D (2000) Ten years of presymptomatic testing for Huntington's disease: the experience of the UK Huntington's Disease Prediction Consortium. J Med Genet 37(8):567–571

Harrington DL, Smith MM, Zhang Y et al (2012) Cognitive domains that predict time to diagnosis in prodromal Huntington disease. J Neurol Neurosurg Psychiatry 83(6):612–619

Huntington G (1872) On chorea. Med Surg Report: Weekly J 26(15):317–321

Huntington Study Group (1996) Unified Huntington's Disease Rating Scale: reliability and consistency. Mov Disord 11(2):136–142

Huntington Study Group (2006) Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. Neurology 66(3):366–372

International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea (1994) Neurology Guidelines for the molecular genetics predictive test in Huntington's disease. 44(8):1533-6

Killoran A, Biglan KM, Jankovic J et al (2013) Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS. Neurology 80(22):2022–2027

Kirkwood SC, Siemers E, Stout JC et al (1999) Longitudinal cognitive and motor changes among presymptomatic Huntington disease gene carriers. Arch Neurol 56(5):563–568

Kremer B (2002) Clinical Neurology of Huntington's disease. In: Bates GP, Harper P, Jones L (eds) Huntington's disease, 3rd edn. Oxford University Press, Oxford, pp 28–53
Kremer B, Almqvist E, Theilmann J et al (1995) Sex-dependent mechanisms for expansions and contractions of the CAG repeat on affected Huntington disease chromosomes. Am J Hum Genet 57(2):343–350
Langbehn DR, Brinkman RR, Falush D et al (2004) A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clin Genet 65(4):267–277
Langbehn DR, Hayden MR, Paulsen JS et al (2010) CAG-repeat length and the age of onset in Huntington disease (HD): a review and validation study of statistical approaches. Am J Med Genet B Neuropsychiatr Genet 153B(2):397–408
Lanska DJ, Lanska MJ, Lavine L et al (1988) Conditions associated with Huntington's disease at death. A case-control study. Arch Neurol 45(8):878–880
Lipe H, Schultz A, Bird TD (1993) Risk factors for suicide in Huntingtons disease: a retrospective case controlled study. Am J Med Genet 48(4):231–233
Loy CT, McCusker EA (2013) Is a motor criterion essential for the diagnosis of clinical huntington disease? PLoS Curr. doi:10.1371/currents.hd.f4c66bd51e8db11f55e1701af937a419
Mason SL, Barker RA (2009) Emerging drug therapies in Huntington's disease. Expert Opin Emerg Drugs 14(2):273–297
Myers RH, Sax DS, Koroshetz WJ et al (1991) Factors associated with slow progression in Huntington's disease. Arch Neurol 48(8):800–804
Novak MJ, Tabrizi SJ (2010) Huntington's disease. BMJ 340:c3109
Orth M, Handley OJ, Schwenke C et al (2010) Observing Huntington's Disease: the European Huntington's Disease Network's REGISTRY. PLoS Curr. doi:10.1371/currents.RRN1184
Orth M, Handley OJ, Schwenke C et al (2011) Observing Huntington's disease: the European Huntington's Disease Network's REGISTRY. J Neurol Neurosurg Psychiatry 82(12):1409–1412
Paulsen JS, Ready RE, Hamilton JM et al (2001a) Neuropsychiatric aspects of Huntington's disease. J Neurol Neurosurg Psychiatry 71(3):310–314
Paulsen JS, Zhao H, Stout JC et al (2001b) Clinical markers of early disease in persons near onset of Huntington's disease. Neurology 57(4):658–662
Paulsen JS, Nehl C, Hoth KF et al (2005) Depression and stages of Huntington's disease. J Neuropsychiatry Clin Neurosci 17(4):496–502
Paulsen JS, Hoth KF, Nehl C et al (2005b) Critical periods of suicide risk in Huntington's disease. Am J Psychiatry 162(4):725–731
Paulsen JS, Langbehn DR, Stout JC et al (2008) Detection of Huntington's disease decades before diagnosis: the Predict-HD study. J Neurol Neurosurg Psychiatry 79(8):874–880
Penney JB, Young AB, Shoulson I et al (1990) Huntington's disease in Venezuela: 7 years of follow-up on symptomatic and asymptomatic individuals. Mov Disord 5(2):93–99
Rawlins M (2010) Huntington's disease out of the closet? Lancet 376(9750):1372–1373
Roos RA (2010) Huntington's disease: a clinical review. Orphanet J Rare Dis 5(1):40
Rosenblatt A, Leroi I (2000) Neuropsychiatry of Huntington's disease and other basal ganglia disorders. Psychosomatics 41(1):24–30
Ross CA, Tabrizi SJ (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 10(1):83–98
Rothlind JC, Bylsma FW, Peyser C et al (1993) Cognitive and motor correlates of everyday functioning in early Huntington's disease. J Nerv Ment Dis 181(3):194–199
Rubinsztein DC, Leggo J, Coles R et al (1996) Phenotypic characterization of individuals with 30–40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36–39 repeats. Am J Hum Genet 59(1):16–22
Shoulson I, Chase TN (1975) Huntington's disease. Annu Rev Med 26:419–436
Shoulson I, Fahn S (1979) Huntington disease: clinical care and evaluation. Neurology 29(1):1–3
Siemers E, Foroud T, Bill DJ et al (1996) Motor changes in presymptomatic Huntington disease gene carriers. Arch Neurol 53(6):487–492

Siesling S, Vegter-van de Vlis M, Losekoot M et al (2000) Family history and DNA analysis in patients with suspected Huntington's disease. J Neurol Neurosurg Psychiatry 69(1):54–59
Snell RG, MacMillan JC, Cheadle JP et al (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nat Genet 4(4):393–397
Snowden JS, Craufurd D, Thompson J et al (2002) Psychomotor, executive, and memory function in preclinical Huntington's disease. J Clin Exp Neuropsychol 24(2):133–145
Tabrizi SJ, Langbehn DR, Leavitt BR et al (2009) Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet Neurol 8(9):791–801
Tabrizi SJ, Scahill RI, Durr A et al (2011) Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. Lancet Neurol 10(1):31–42
Tabrizi SJ, Reilmann R, Roos RA et al (2012) Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. Lancet Neurol 11(1):42–53
Tabrizi SJ, Scahill RI, Owen G et al (2013) Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol 12(7):637–649
The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72(6):971–983
van der Burg JM, Björkqvist M, Brundin P (2009) Beyond the brain: widespread pathology in Huntington's disease. Lancet Neurol 8(8):765–774
van der Steenstraten IM, Tibben A, Roos RA et al (1994) Predictive testing for Huntington disease: nonparticipants compared with participants in the Dutch program. Am J Hum Genet 55(4):618–625
van Duijn E, Kingma EM, van der Mast RC (2007) Psychopathology in verified Huntington's disease gene carriers. J Neuropsychiatry Clin Neurosci 19(4):441–448
Videnovic A, Leurgans S, Fan W et al (2009) Daytime somnolence and nocturnal sleep disturbances in Huntington disease. Parkinsonism Relat Disord 15(6):471–474
Went L (1990) Ethical issues policy statement on Huntington's disease molecular genetics predictive test. International Huntington Association. World Federation of Neurology. J Med Genet 27(1):34–38
Wexler A (2010) Stigma, history, and Huntington's disease. Lancet 376(9734):18–19
Wexler NS, Lorimer J, Porter J et al (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 101(10):3498–3503
Wild EJ, Tabrizi SJ (2007) The differential diagnosis of chorea. Pract Neurol 7(6):360–373
Wild EJ, Mudanohwo EE, Sweeney MG et al (2008) Huntington's disease phenocopies are clinically and genetically heterogeneous. Mov Disord 23(5):716–720
Zühlke C, Riess O, Bockel B et al (1993) Mitotic stability and meiotic variability of the (CAG)n repeat in the Huntington disease gene. Hum Mol Genet 2(12):2063–2067

The Neuropathology of Huntington's Disease

Henry J. Waldvogel, Eric H. Kim, Lynette J. Tippett, Jean-Paul G. Vonsattel and Richard LM Faull

Abstract The basal ganglia are a highly interconnected set of subcortical nuclei and major atrophy in one or more regions may have major effects on other regions of the brain. Therefore, the striatum which is preferentially degenerated and receives projections from the entire cortex also affects the regions to which it targets, especially the globus pallidus and substantia nigra pars reticulata. Additionally, the cerebral cortex is itself severely affected as are many other regions of the brain, especially in more advanced cases. The cell loss in the basal ganglia and the cerebral cortex is extensive. The most important new findings in Huntington's disease pathology is the highly variable nature of the degeneration in the brain. Most interestingly, this variable pattern of pathology appears to reflect the highly variable symptomatology of cases with Huntington's disease even among cases possessing the same number of CAG repeats.

Keywords Human brain · Neuropathology · Neurochemical · Striosomes · Basal ganglia · Striatum · Globus pallidus · Symptomatology

H.J. Waldvogel (✉) · E.H. Kim · R.L. Faull
Centre for Brain Research, Department of Anatomy with Radiology, University of Auckland, Auckland, New Zealand
e-mail: h.waldvogel@auckland.ac.nz

E.H. Kim
e-mail: e.kim@auckland.ac.nz

R.L. Faull
e-mail: rlm.faull@auckland.ac.nz

J.-P.G. Vonsattel
Department of Pathology, Presbyterian Hospital, Columbia University, New York NY10032, USA
e-mail: jgv2001@columbia.edu

L.J. Tippett
Centre for Brain Research, Department of Psychology, University of Auckland, Auckland, New Zealand
e-mail: l.tippett@auckland.ac.nz

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1 Introduction

This chapter provides an update of the current knowledge of neuropathological changes that occur in the human brain in Huntington's disease (HD), and also an outlook to future studies in human HD neuroanatomy. A HD brain may be about 20–30 % less than a control brain in weight although this will be variable depending on the severity of the disease (Vonsattel and DiFiglia 1998). Major pathology occurs most prominently in the neostriatum which includes the caudate nucleus and putamen but also other regions of the basal ganglia. The basal ganglia are a highly interconnected set of subcortical nuclei and so major atrophy in one or more regions have major effects on the others. Therefore, the striatum which is preferentially degenerated and receives projections from the entire cortex also affects the regions to which it targets especially the globus pallidus and substantia nigra pars reticulata. Additionally, the cerebral cortex is itself severely affected as are many other regions of the brain, especially in more advanced cases. The most important new findings in

HD pathology are the highly variable nature of the degeneration in the brain (Hadzi et al. 2012; Pillai et al. 2012). For instance, there is an overall 21–29 % loss in cross-sectional area of the cerebral cortex but 57 % loss in the caudate nucleus (de la Monte et al. 1988). As discussed later, the cell loss in the cerebral cortex also shows a high degree of variability. Most interestingly, this variable pattern of pathology appears to reflect the highly variable symptomatology of cases with HD even among cases possessing the same number of CAG repeats (Georgiou et al. 1999; Friedman et al. 2005; Gomez-Esteban et al. 2007; Tippett et al. 2007; Thu et al. 2010).

2 Symptomatology

What has been known for a long time is that cases affected by HD express a triad of symptoms which include motor, behavioral, and cognitive deficits, although the defining symptom has always remained that of chorea. However, despite the single-gene etiology of HD, there is remarkable variability in the types of these motor, behavioral, and cognitive symptoms present in different HD cases both at clinical onset, during the disease, and at end stage of the disease. It must be remembered that the vast majority of pathological studies carried out on postmortem HD human brain are at end stage of the disease.

During the lifetime of individuals with HD, some exhibit mainly motor dysfunction at clinical onset, and few if any changes in mood for extended periods of time while, at the other extreme, others show mainly mood and/or cognitive changes, with minimal involuntary movements until the late stages of the disease (Andrew et al. 1993; Claes et al. 1995). Still others experience marked motor, mood, and cognitive symptoms simultaneously (Brandt and Butters 1986; Folstein 1989; Myers et al. 1991; Claes et al. 1995; Zappacosta et al. 1996; Thompson et al. 2002). Interestingly, observations in monozygotic twins who inherited identical *HTT* genes with the same repeat length exhibit marked differences in their symptom profile (Georgiou et al. 1999; Friedman et al. 2005; Gomez-Esteban et al. 2007). The onset of clinical symptoms in individual HD cases is generally correlated with the number of CAG repeats (Wexler et al. 2004), as does the disease severity, but there is no consistent relationship between CAG repeat length and symptom subtype (MacMillan et al. 1993; Telenius et al. 1994; Claes et al. 1995; Zappacosta et al. 1996). Thus, the source of variability in symptom subtypes is not clear. The clinical diagnosis and the onset of the disease are generally based on the onset of the movement disorder termed Huntington's chorea. These characteristic motor symptoms are expressed as a severe "choreoathetotic" disorder, which describes the rapid, irregular, and involuntary movements of HD. In addition, clumsiness and unsteadiness in walking are also early symptoms. Studies on HD populations have indicated that approximately 50–70 % of cases at onset present with chorea (Di Maio et al. 1993; Witjes-Ane et al. 2002) and chorea may develop into rigidity and dystonia later in the disease. However, 30–50 % present first, most commonly with depression followed by cognitive and behavioral changes and emotional problems such as irritability, aggression, anxiety,

and obsessive behavior (Di Maio et al. 1993; Witjes-Ane et al. 2002). There is also considerable phenotypic variation in the pattern of symptomatology during the course of the disease. To be able to more successfully grade HD clinically, several rating scales for HD have been developed, for instance, the Quantitated Neurological Exam (QNE), the HD Functional Capacity Scale (HDFCS), and the HD Motor Rating Scale (HDMRS). Recently, a new combined scale was developed by the Huntington's Study Group to include the four domains in HD: motor function, cognitive function, behavioral abnormalities, and functional capacities and is termed The Unified Huntington's Disease Rating Scale (UHDRS), which aims to be suitable for tracking changes over time (Huntington Study Group 1996).

3 Pathology in the Basal Ganglia

3.1 Basal Ganglia Organization

The basal ganglia are a group of large nuclei located subcortically in the base of the forebrain and are involved with the control of mood and movement. The nuclei belonging to the basal ganglia were originally considered to be the principal components of the "extrapyramidal system" and by convention, the term basal ganglia is now restricted mainly to the striatum (comprised of the caudate nucleus and putamen), globus pallidus segments, the subthalamic nucleus (STN), and the substantia nigra (Carpenter et al. 1976; Smith et al. 1998). The striatum is divided into the two large nuclear masses, the caudate nucleus, which rostrally forms a head, more centrally a body, and posteriorly a tail region which extends dorsally over the thalamus, and the putamen. The caudate nucleus and putamen are separated by the condensed fibers of the internal capsule. The globus pallidus is divided into two parts: the external segment of the globus pallidus (GPe) and the internal segment of the globus pallidus (GPi) (also termed medial and lateral segments). The STN is located medial to the GPi and rostro-dorsal to the substantia nigra. The substantia nigra consists of two parts, the substantia nigra pars reticulata (SNr), which is located ventrally in the midbrain, and the substantia nigra pars compacta (SNc), which in humans and primates are pigmented, and is located as cell clusters in the dorsal regions of the substantia nigra. Although the substantia nigra is located in the midbrain, it is considered part of the basal ganglia due to its close functional and connectional interrelationships with the striatum.

The striatum comprises neurons that fall principally into two classes of neurons —projection neurons and local circuit neurons—and these are subclassified according to their size, neurochemistry, and connectional characteristics. The majority of these neurons (approximately 95 %) are the medium spiny projection neurons using the inhibitory neurotransmitter γ-aminobutyric acid (GABA), which project mainly to the globus pallidus and SNr, and the remaining neurons are a morphologically and neurochemically heterogeneous group of interneurons, which modulate the function of the medium spiny output neurons (see below).

3.2 Basal Ganglia Pathways

The basal ganglia are integrated into a circular interconnected forebrain loop, which forms a cortical/basal ganglia/thalamus/cortical circuit (Nauta and Domesick 1984), (see Fig. 1). The cortex provides a major excitatory glutamatergic input to the caudate nucleus and putamen (Carpenter et al. 1976) that arises bilaterally but with a predominant ipsilateral component from the entire cerebral cortex with a major projection from the sensorimotor cortex (McGeorge and Faull 1989). The projection from the cerebral cortex to the striatum forms the single most extensive afferent connection to the striatum; practically every cortical region projects to the striatum (McGeorge and Faull 1989). In addition, the caudate-putamen receives projections from regions other than the cortex; an excitatory projection from the intralaminar nuclei and other nuclei of the thalamus (Sadikot et al. 1992), an inhibitory feedback loop from the globus pallidus (Bevan et al. 1998), a major dopaminergic projection from the SNc (A9 cell group) plus other afferent connections from diverse nuclei such as the serotonergic dorsal raphe nucleus (Graybiel et al. 1979) and cholinergic and glutamatergic projections from the pedunculopontine nucleus in the midbrain (Mena-Segovia et al. 2004). The main flow of cortical information through the basal ganglia is based on what are termed the "direct" or "indirect" pathways (Albin et al. 1989; Alexander and Crutcher 1990; DeLong 1990; Parent and Hazrati 1993; Graybiel 1995; Yung et al. 1996; Smith et al. 1998), and these are critical to our understanding of HD pathophysiology (see Fig. 1). According to this model, cortical information, which flows to the striatum, is processed and transmitted through the basal ganglia via two routes. First, a direct GABAergic inhibitory pathway flows from the striatum to the GPi and the SNr (*direct pathway*) which also contains the co-transmitter substance P. Secondly, an output from the striatum containing enkephalin projects to the external segment of the GPe, which in turn, sends an inhibitory input to the STN which in turn sends an excitatory projection to the GPi (*indirect pathway*). Thus, the direct and indirect pathways converge on the GPi which provides an inhibitory output projection to the ventral anterior and ventral lateral (VA/VL) nuclear regions of the thalamus. The VA/VL thalamic nucleus that receives most of the input from the GPi and SNr projects an excitatory input mainly to the frontal and premotor cortex (Mehler 1971; Faull and Mehler 1978; Kayahara and Nakano 1996) which then influences the output from the motor cortex. This completes the cerebro-cortical/basal ganglia/thalamus/cortical circuit. This circuit converges on the output of the primary motor cortex and intimately controls the movement of muscles that is critically affected in HD. In addition, a recent pathway termed "the hyperdirect pathway" has been identified which is an excitatory link from the cerebral cortex directly to the STN, which can stimulate subthalamic neurons to give a powerful excitatory drive to the GPi output neurons which inhibit the thalamus, and in this way bypass the striatum, see Fig. 1 (Nambu et al. 2002).

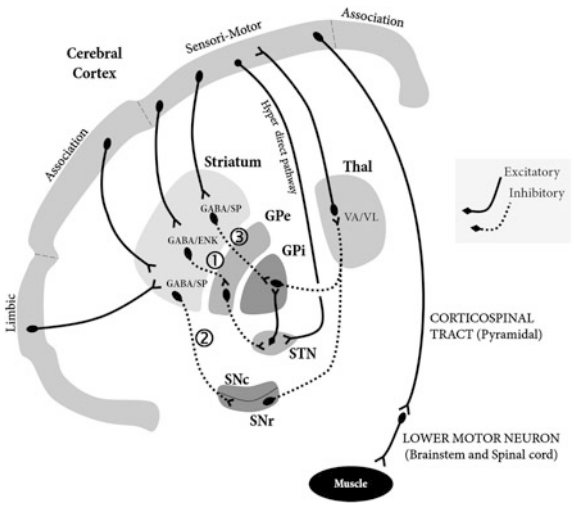


Fig. 1 Schematic diagram of cortico-basal ganglia pathways in Huntington's disease. The projections in the cortico-basal ganglia-thalamo-cortical loop form several functionally segregated parallel and interconnected systems. Prominent among these are the motor circuit, which involves the motor and premotor cortices and the dorsal striatum. In the *indirect pathway* (①), the excitatory corticostriatal projection terminates onto striatal medium spiny neurons that contain GABA/ENK. This striatal output first passes to the inhibitory GPe and then via the excitatory STN to GPi whereby disinhibition of the subthalamic neurons result in excitation of the GPi and hence **inhibition of the VA-VL thalamic nuclei**. In the *direct pathway* (②, ③), the cortical excitatory fibers terminate on the medium spiny striatal projection neurons that contain GABA/SP which project to GPi and SNr. These result in inhibition of the GPi and SNr and disinhibition **(i.e., excitation) of the VA/VL thalamic** output to the cerebral cortex. Thus, the result of cortical activation in the direct pathway is opposite to that of the indirect circuit: reinforcement rather than reduction of cortical activity. The hyperdirect pathway originates from the motor regions of the cerebral cortex and terminates in the STN and provides a direct excitatory pathway from the cerebral cortex to the STN. The disruption of the excitatory glutaminergic projection onto the striatum results in dysfunction of the striatal output pathways in HD which ultimately leads to the development of motor dysfunction in both hyperkinetic and dyskinetic movements. In HD, the initial symptoms of hyperkinesia and chorea are caused by the initial preferential damage in the *indirect* GABA/ENK striatopallidal pathway (①) that project from the striatum to the GPe. The loss of striatal neurons that give rise to the indirect pathway reduces the inhibitory action on the GPe which increases inhibition on the STN. The STN then becomes hypofunctional and causes reduced excitation of the inhibitory action of the GPi upon the thalamus. This subsequent disinhibition of the thalamus leads to the overactivation of the motor cortex which results in chorea (hyperactivity). By contrast, the subsequent later loss of *direct* GABA/SP striatopallidal pathway (②, ③) that projects from the striatum to the GPi and SNr causes increased inhibition of the thalamus which decreases the activation of the motor cortex with resultant rigidity (hypoactivity) in the later stages of the disease. The continuous and *dotted lines* indicate excitatory and inhibitory pathways, respectively. *ENK* enkephalin, *GABA* γ-aminobutyric acid, *GPe* globus pallidus external segment, *GPi* globus pallidus internal segment, *SNc* substantia nigra pars compacta, *SNr* substantia nigra pars reticulata, *SP* substance P, *STN* subthalamic nucleus, *Thal* thalamus, and *VA/VL* ventral anterior/ventral lateral thalamic nuclei

4 Neuropathology of the Basal Ganglia

4.1 Macroscopic Changes

Gross examination of postmortem Huntington's diseased human brain demonstrates a striking characteristic bilateral atrophy of the striatum (de la Monte et al. 1988; Aylward et al. 1997; Vonsattel and DiFiglia 1998; Vonsattel et al. 2008). This degeneration generally follows an ordered and topographical distribution. The tail and body of the caudate nucleus show more degeneration than the head in the very early stages of the degenerative process. The pattern of degeneration in the caudate nucleus and the putamen usually progresses from the tail of the caudate nucleus (TCN) to the head and body in the caudo-rostral and simultaneously in the dorsoventral and medio-lateral directions (Vonsattel and DiFiglia 1998).

Macroscopically, the volume of the caudate nucleus and putamen is reduced with subsequent alteration of their respective shape (Fig. 2). A study of 30 HD brains showed the putamen had an average 64 % cross-sectional area loss compared with a 57 % cross-sectional area loss in the caudate nucleus (de la Monte et al. 1988). With progression of HD, the caudate nucleus becomes progressively more atrophic changing from the characteristic normal convex shape defining the border of the lateral ventricle to a thinner and ultimately more concave shape with resultant enlargement of the lateral ventricles occurring in parallel. This gradually decreasing volume is due to the loss of especially the medium spiny neurons, and their dendritic arbors and heavily myelinated axonal projections. Combined with the neuronal degeneration, there is also marked gliosis by astrocytes and oligodendrocytes. The extent of the macroscopic shape of the caudate nucleus and associated ventricular enlargement, the microscopic striatal degeneration including the loss of striatal neurons, and extent of gliosis provides the basis of the Vonsattel-grading system which is detailed below (Vonsattel et al. 1985; Vonsattel and DiFiglia 1998).

More recently, studies carried out by in vivo neuroimaging of brains of HD patients have detected early changes in the volume and shape of the basal ganglia, cerebral cortex, and other regions, and these were evident several years prior to symptomatic onset (Reading et al. 2005; Rosas et al. 2005).

4.2 Grading of Striatal Neuropathology

Although the degenerative process occurring in HD gradually encompasses the entire brain with regional differential degree of severity, most studies have emphasized that the brunt of the slowly ongoing atrophy involves the neostriatum. As previously stated, the neostriatal neuronal loss and reactive gliosis have an ordered and topographic distribution (Kiesselbach 1914; Lewy 1923; Terplan 1924; Dunlap 1927; Schroeder 1931; Neustaedter 1933; Birnbaum 1941; Hallervorden 1957; McCaughey 1961; Forno and Jose 1973; Roos et al. 1985; Vonsattel et al. 1985).

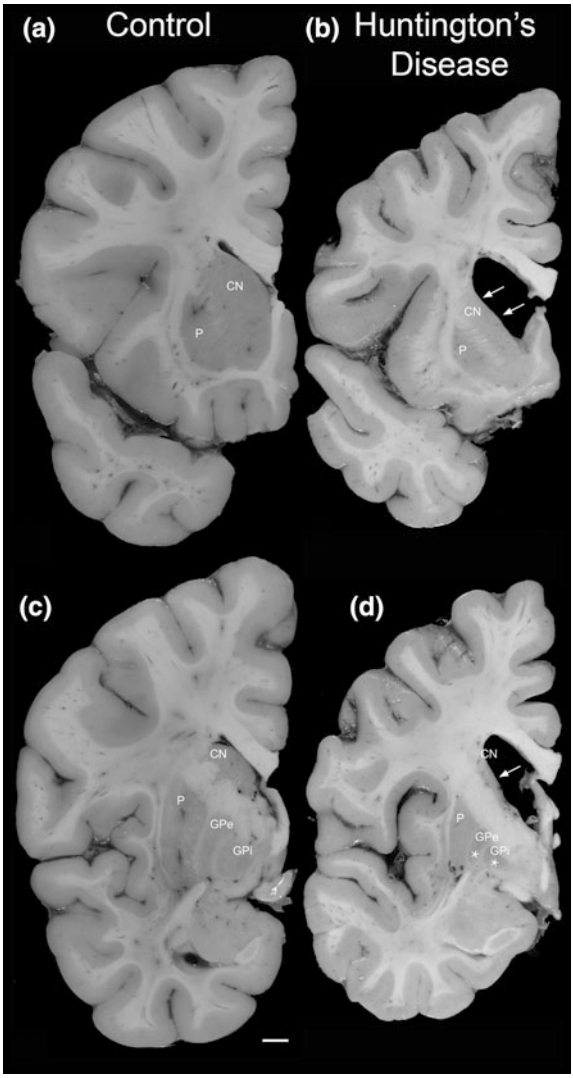


Fig. 2 Pathology of Huntington's diseased brain. Coronal sections at 2 levels through the human brain of **a**, **c** a representative control case the left cerebral hemisphere of a 35-year-old male, and **b**, **d** a Grade 3/4 Huntington's disease case. **a**, **b** are from the level of the striatum and the nucleus accumbens, **c**, **d** are at the level of the globus pallidus. There is major shrinkage of the caudate nucleus and putamen (*arrows*) as well as the globus pallidus (*asterisks*) in the Huntington's disease case. Shrinkage of the cerebral cortex is also evident. *CN* caudate nucleus, *GPe* globus pallidus external segment, *GPi* globus pallidus internal segment and *P* putamen; Scale bar = 1 cm

Along the sagittal axis of the neostriatum, the TCN is more involved than the body (BCN), which in turn is more involved than the head (HCN). The caudal portion of the putamen is more degenerated than the rostral portion; the transition between the portions is gradual, thus often ill defined.

Along the coronal (or dorsoventral) axis of the neostriatum, the dorsal and rostral neostriatal regions are more involved than the ventral ones including the nucleus accumbens. Along the medio-lateral axis (half brain) or latero-lateral axis (whole brain), the paraventricular half of the CN is more involved than the paracapsular half, the transition between the halves being gradual. As a function of the duration

of the deleterious process, neostriatal degeneration appears to simultaneously move in a caudo-rostral direction, in a dorsoventral direction, and in a medio-lateral direction. Fibrillary astrogliosis parallels the loss of neurons along the caudo-rostral and dorsoventral gradients of decreasing severity. Most neostriatal neurons visible in the postmortem brains are apparently normal, although the lipofuscin might be increased or some neurons might be smaller than normally expected. Clearly, a subset of neostriatal neurons stain darker with Luxol fast blue counterstained with hematoxylin and eosin (LHE), or hematoxylin and eosin (HE), or with cresyl violet (CV) than the apparently healthy, but probably dysfunctional neurons. These neurons are referred to as neostriatal dark neurons (NDN). They have a scalloped cellular membrane, a granular dark cytoplasm, and a nucleus with condensed chromatin. They are scarce, but tend to be clustered, in both the atrophic and in the relatively preserved zones. Less than 5 % of the HD brains show discrete, round 0.5–1.0 mm in diameter islets of relatively intact parenchyma within the anterior neostriatum. The density of neurons in islets is the same as, or slightly lower than that of the control neostriatum, but the density of astrocytes is increased (Vonsattel et al. 1992). Islets are found more frequently in cases with juvenile than adult onset of clinical symptoms.

A neuropathological grading system for HD was developed by Vonsattel based on the pattern of neurodegeneration in the HD striatum of a large number of HD brains (Vonsattel et al. 1985; Glass et al. 2000). The assignment of a grade of neuropathological severity from 0 to 4 is based on gross and microscopic findings using conventional methods of examination obtained from standardized, coronal sections that include the striatum: at the level of the nucleus accumbens (Fig. 2a, b), at the level of the caudal edge of the anterior commissure and globus pallidus (Fig. 2c, d), and at the level of the lateral geniculate body. This grading system applies to brains from individuals diagnosed clinically as having HD, with or without a genetic test.

Grade 0 comprises less than 1 % of all HD brains. Gross examination shows features indistinguishable from control brains. On general survey using LHE- or HE-stained slides alone, neither reactive gliosis nor neuronal loss is reliably detectable. However, further evaluations including cell counts indicate a 30–40 % loss of neurons in the HCN and no visible reactive astrocytosis. Furthermore, a study using immunohistochemistry and sections of three, presymptomatic, gene carriers revealed ubiquitinated, nuclear inclusions in all three brains including one individual, with 37 polyQ, who died putatively 3 decades before the expected age for onset of symptoms. In addition, cell counts of the TCN revealed an increased density of oligodendrocytes among the presymptomatic *HTT* gene carriers (Gomez-Tortosa et al. 2001).

Grade 1 comprises 4 % of all HD brains. The TCN is smaller than control as most likely is the BCN. Neuronal loss and astrogliosis involve the TCN, BCN, and dorsal portion of both the head and nearby dorsal putamen. Cell counts show 50 % or greater loss of neurons in the HCN.

Grade 2 comprises 16 %; those assigned **Grade 3** comprises 52 %; and those assigned **Grade 4** comprises 28 % of all HD brains. Gross striatal atrophy is mild to

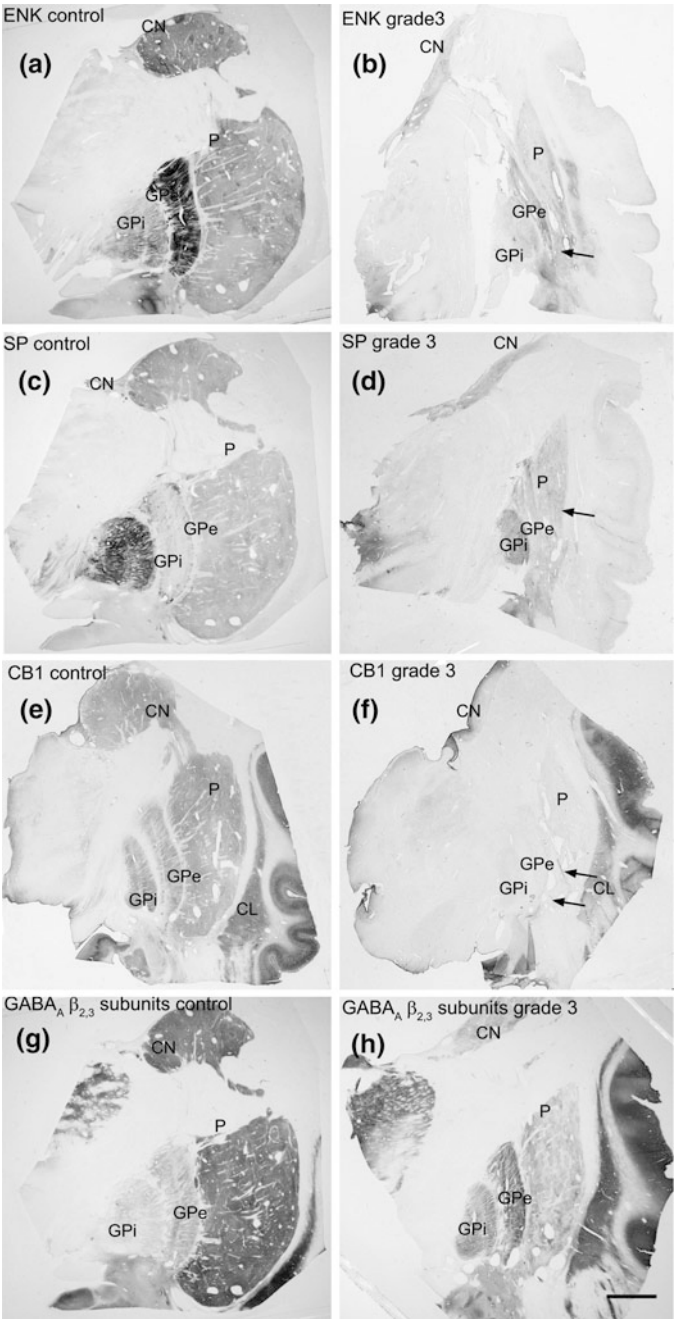
moderate in grade 2 (the medial outline of the HCN is only slightly convex but still bulges into the lateral ventricle) and severe in grade 3 (the medial outline of the HCN forms a straight line or is slightly concave medially). Thus, the microscopical changes in Grade 2 and Grade 3 are more severe than in Grade 1, and less than in Grade 4 brains.

In **Grade 4**, the striatum is severely atrophic (the medial contour of the HCN is concave, as is the CN at the anterior limb of internal capsule). The neostriatum loss is 95 % or more neurons. In at least 50 % of Grade 4 brains, the underlying nucleus accumbens remains relatively preserved, but is not normal.

5 Cellular and Neurochemical Changes

5.1 Striatum

Various autoradiographic, *in situ* hybridization and immunohistochemical studies have documented neuronal and glial changes in the striatum of HD and have reported loss of neurochemicals, neurotransmitters, and neurotransmitter-associated receptors in the HD striatum (Figs. 3, 4, 5; Table 1). The most affected neuronal populations in the HD striatum are the medium-sized spiny projection neurons (MSNs) that constitute ~90–95 % of the total striatal neuronal population. In the human striatum, the cell bodies of these GABAergic inhibitory MSNs can be identified morphologically in histological sections and can also be specifically labeled with antibodies to glutamic acid decarboxylase (GAD, the precursor enzyme for synthesizing GABA), the neuropeptides enkephalin, substance P, dynorphin, and the calcium-binding protein calbindin (CB) (Holt et al. 1996, 1997; Deng et al. 2004) and the dopamine- and cAMP-regulated phosphoprotein 32 kDa, termed DARPP-32. The medium spiny neurons are innervated by excitatory neurons in the cerebral cortex and the thalamus, by dopaminergic neurons in the SNc, and cholinergic and GABAergic interneurons of the striatum. They are therefore associated with a large number of ion channel and metabotropic receptors on their surface membranes including cannabinoid (CB1) (Glass et al. 2000), $GABA_A$ receptors (Waldvogel et al. 1999), glutamate receptors (Dure et al. 1992; Kuppenbender et al. 2000), and dopamine receptors (D1 and D2) (Joyce et al. 1988; Khan et al. 1998). The loss of medium spiny neurons has been shown in immunohistochemical studies using the calcium-binding protein marker CB, which selectively identifies the cell bodies of medium spiny neurons in the matrix compartment (Seto-Ohshima et al. 1988; Goto et al. 1989; Ferrante et al. 1991; Tippett et al. 2007). A recent study showed a loss of 58–76 % DARPP-32 positive neurons in the human HD putamen with increasing grade (Guo et al. 2012). DARPP-32 is a marker for the majority of medium spiny neurons in the rat striatum (Ouimet et al. 1998). The loss of DARPP32 neurons was correlated with the motor impairment score rather than chorea. As mentioned above, there are two major GABAergic populations of MSNs, those that contain


ENK control
(a)
CN
P
GPe
GPi
ENK grade3
(b)
CN
P
GPe
GPi
SP control
(c)
CN
P
GPe
GPi
SP grade 3
(d)
CN
P
GPe
GPi
CB1 control
(e)
CN
P
GPe
GPi
CL
CB1 grade 3
(f)
CN
P
GPe
GPi
CL
GABA$_A$ $\beta_{2,3}$ subunits control
(g)
CN
P
GPe
GPi
GABA$_A$ $\beta_{2,3}$ subunits grade 3
(h)
CN
P
GPe
GPi

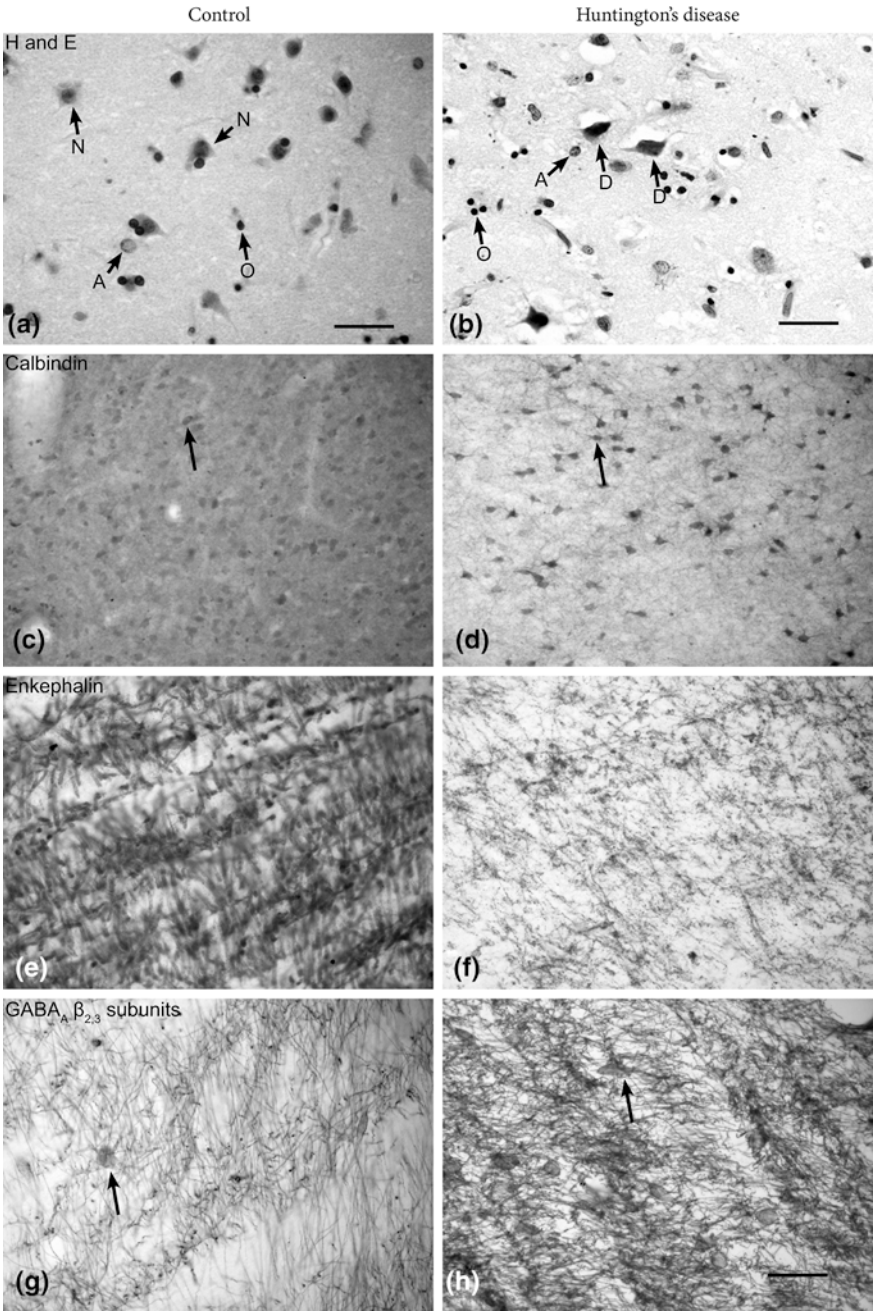
◀ **Fig. 3** Changes in neurochemical markers enkephalin, substance P, cannabinoid CB1, $GABA_A$ receptor α_1 subunit, in the basal ganglia in Huntington's disease human brain. **a**, **c**, **e**, **g** Sections of the control human basal ganglia at the level of the globus pallidus. **b**, **d**, **f**, **h** Sections from Grade 3 Huntington's disease brains at equivalent levels through the globus pallidus. Sections were stained for the following markers: Enkephalin (**a**, **b**), Substance P (**c**, **d**), Cannabinoid CB1 receptor (**e**, **f**), $\beta_{2,3}$ subunit of the $GABA_A$ receptor (**g**, **h**). In Huntington's disease, there is a major loss of enkephalin in the caudate nucleus, putamen, and GPe. There is also a loss of substance P in the caudate nucleus and putamen and GPi, although the loss is not as marked as that of enkephalin and there is an almost complete loss of CB1 receptors in the caudate nucleus and putamen and both the GPe and GPi. Note for CB1 receptor the adjacent claustrum and insular cortex still show relatively normal levels of staining. There is a major upregulation of the $GABA_A$ $\beta_{2,3}$ receptor subunits in both GPe and GPi in Huntington's disease, but a loss in the putamen. *Arrows* indicate globus pallidus in **b**, **f**. (Modified from Allen et al. 2009) *CL* claustrum, *CN* caudate nucleus, *ENK* enkephalin, *GPe* globus pallidus external segment, *GPi* globus pallidus internal segment, *P* putamen, *SP* substance P, and Scale bar = 1 cm

enkephalin, and those that contain substance P (see Figs. 1 and 5). In HD, the MSNs degenerate with increasing HD grade (Vonsattel et al. 1985; Vonsattel and DiFiglia 1998). Both enkephalin and substance P neurons are lost (Marshall et al. 1983), but MSNs projecting to the external segment of the GP (indirect pathway) that express enkephalin and dopamine D2 receptors have been shown to be the most vulnerable in the disease process (Reiner et al. 1988; Albin et al. 1992; Augood et al. 1996), and degenerate in advance of the MSNs that express substance P, dynorphin and dopamine D1 receptors that project to the GPi and SNr (direct pathway) (Gerfen et al. 1990; Deng et al. 2004). The disruption of these striatal pathways in HD leads to the development of motor dysfunction including hyperkinetic, hypokinetic, and dyskinetic movements (see Figs. 1 and 5).

Reductions in glutamate NMDA receptor binding, $GABA_A$ receptor binding, and cannabinoid receptor binding are all evident in the HD striatum (Whitehouse et al. 1985; Young et al. 1988; Glass et al. 2000; Tippett et al. 2007), which is most likely due to loss of neurons containing these receptors, but may also represent a dysfunction or downregulation of these receptors.

The projections from the striatum to the output nuclei which contain enkephalin, substance P, and cannabinoid receptors are progressively lost with increasing grade, mirroring the loss of MSNs in the striatum (Figs. 13a–d and 5). Enkephalin staining is dramatically lost in a grade-dependent manner in the GPe reflecting loss of the GABAergic enkephalin-positive pathway to the GPe (indirect pathway) (Figs. 3a, b, and 5). There is also a loss of substance P in the GPi (Figs. 3c, d, and 5) and SNr, reflecting the loss of the GABAergic substance P-positive pathway projecting to these two striatal output nuclei (Reiner et al. 1988; Waters et al. 1988; Albin et al. 1990, 1992; Deng et al. 2004; Allen et al. 2009), and loss of cannabinoid receptors on the presynaptic terminals of both the direct and indirect pathways (Figs. 3e, f and 5).

In addition to the projection neurons, the striatum contains a heterogeneous group of aspiny interneurons, which modulate the activity of medium spiny neurons in a highly complex fashion (Cicchetti et al. 2000). The majority of interneurons contain GABA as their major neurotransmitter, and these are subdivided into groups depending on the calcium-binding proteins they contain, principally


Control
Huntington's disease
H and E
N
N
A
O
(a)
A
D
D
O
(b)
Calbindin
(c)
(d)
Enkephalin
(e)
(f)
GABA$_A$ $\beta_{2,3}$ subunits
(g)
(h)

◀**Fig. 4** Striatal neurons in control and Huntington's disease striatum. Examples of histochemically labeled striatal projection neurons and interneurons in the control striatum (**a**, **c**, **e**, **g**) and Grade 3 Huntington's disease striatum (**b**, **d**, **f**, **h**) from equivalent regions of the striatum. **a**, **b** Hematoxylin- and eosin-stained sections in the region of the (**a**) control human dorsal striatum showing neurons resembling normal medium-sized spiny neurons, astrocytes and oligodendrocytes and (**b**) Huntington's diseased human dorsal striatum showing atrophic neurons, astrocytes, and oligodendrocytes: For **a** and **b**: *arrow*—A = astrocyte, *arrow*—D = degenerating neuron, *arrow*—N = normal neuron, *arrow*—O = oligodendrocytes. **c**, **d** Medium spiny neurons stained with calbindin from a control case (**c**) and a Grade 3 HD case (**d**) showing marked loss of calbindin-positive neurons and neuropil. **e**, **f** Enkephalin staining of axon terminals in a control GPe (**e**) compared with an HD Grade 3 case showing loss of enkephalin-positive terminal staining on pallidal dendrites (**f**) in the HD case. **g**, **h** (**G**) Illustrates $GABA_A$ receptor $\beta_{2,3}$ subunit staining on pallidal neurons and dendrites in the control GPe from the same case as **e** compared with **h** the same HD Grade 3 case as in **f** showing that associated with the loss of enkephalin terminals there is upregulation of these subunits on pallidal neurons and dendrites in the HD GPe. Scale bars **a**, **b** = 25 μm **h** represents scale bar for **c**–**h** = 100 μm

parvalbumin (PV) and calretinin (CR) (Cicchetti et al. 2000). The two other major types of interneurons in the human striatum are the large cholinergic interneurons, which also contain CR and substance P receptors, and the interneurons containing neuropeptide Y, somatostatin, NOS, and NADPH diaphorase (see Figs. 3 and 5).

In general, the striatal interneurons are less affected by the disease process than the medium spiny neurons, especially in lower grades (Ferrante et al. 1987); however, in higher grades, the interneurons are also affected in a differential manner; that is, those containing the calcium-binding protein PV (Harrington and Kowall 1991; Reiner et al. 2013) are consistently lost in the HD striatum, and a recent study shows that the PV-positive fast-spiking interneurons of the striatum are degenerated in a grade-dependent manner, so that by Grade 3, they are severely reduced in number, have a compromised morphology and that they may be linked to those patients developing dystonia (Reiner et al. 2013). The large-sized CR-positive neurons many of which belong to the population of the large-sized cholinergic neurons are generally preserved until the higher grades (Cicchetti and Parent 1996). By contrast, interneurons containing neuropeptide Y/somatostatin or NADPH diaphorase/NOS and the medium-sized CR-positive interneurons are largely spared even in relatively severe cases of striatal degeneration (Dawbarn et al. 1985; Ferrante et al. 1987; Cicchetti and Parent 1996; Cicchetti et al. 2000). The reason for this is not clear, but it has been postulated to be due to either, the pattern of distribution of excitatory receptors, the presence of differential types of calcium-binding proteins which buffer toxic intracellular calcium concentrations, or possibly their genetic fingerprint and susceptibility to the toxic mutant *HTT* gene. The calcium-binding protein CB is however not considered neuroprotective as the MSNs are preferentially affected from the earliest stages of the disease and show massive cell loss. However, the CR-positive medium-sized neurons may be protected by the calcium-binding protein CR, as these are largely preserved in HD.

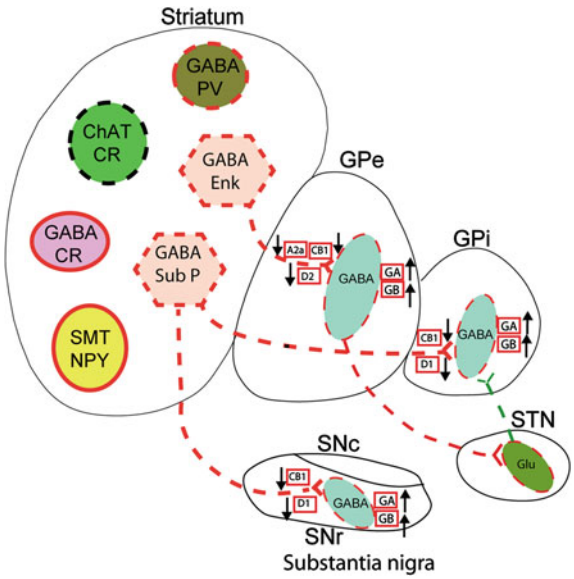


Fig. 5 Cell and receptor changes in the basal ganglia in Huntington's disease. Diagrammatic figure showing the various cell types and receptors in the striatum, globus pallidus external segment (*GPe*), globus pallidus internal segment (*GPi*), substantia nigra (*SN*), and subthalamic nucleus (*STN*). In the striatum, the GABAergic medium spiny projection neurons are divided into 2 groups, those that stain for enkephalin (*Enk*) and those staining for substance P (*Sub P*): These project to the large GABAergic pallidal neurons in the GPe and the GPi and to GABAergic projection neurons in the SNr, respectively. The small boxes near the axon terminals in the GPe and GPi represent the presynaptic A2a adenosine, D1 or D2 dopamine receptors and CB1 cannabinoid receptors. The boxes on the pallidal cells represent postsynaptic $GABA_A$ receptors (*GA*) and $GABA_B$ receptors (*GB*). The four remaining populations in the striatum are those representing GABAergic interneurons staining for parvalbumin (*PV*), calretinin (*CR*), choline acetyl transferase (*ChAT*) and calretinin, neuropeptide Y (*NPY*), and somatostatin (*SMT*). In Huntington's disease, neurons that degenerate are indicated by dashed lines. These include the medium-sized GABAergic spiny projection neurons containing Enk and Sub P, the interneurons containing parvalbumin and interneurons containing ChAT. Those interneurons containing only calretinin and those containing NPY/SMT are relatively spared. Neurons in the output nuclei are also lost, those in the GPe, GPi, and STN. *Arrows* indicate upregulation or downregulation of receptors in the output nuclei in HD. The GABA/Enk striato-GPe neurons and their associated receptors are affected in the early stages of the disease while the GABA/Sub P (striato-GPi, striato-SNr) neurons and receptors are affected in HD cases with more advanced pathology. *GPe* globus pallidus external segment, *GPi* globus pallidus internal segment, *STN* subthalamic nucleus, *SNr* substantia nigra pars reticulata, and *SNc* substantia nigra pars compacta

5.2 Striosome-matrix Compartmental Degeneration in the Striatum and Its Relation to Symptom Profile

The mammalian striatum is further subdivided into two major interdigitating compartments: The smaller neurochemicallyly defined islands termed striosomes and the surrounding extrastriosomal region termed the matrix. These compartments

Table 1 Neurochemical changes in the various nuclei in the basal ganglia of the human brain in Huntington's disease

Neurochemical	Region	References
Calbindin	Striatum↓ GPe↓ GPi↓	Seto-Ohshima et al. (1988), Tippett et al. (2007)
Calretinin	Striatum↑	Cicchetti and Parent (1996)
Cannabinoid receptors	Striatum↓	Glass et al. (1993), Richfield and Herkenham (1994), Allen et al. (2009)
Dopamine receptors		
D1	Striatum↓	Reisine et al. (1978), Joyce et al. (1988), Richfield et al. (1991), Weeks et al. (1996)
D2	Striatum↓	Reisine et al. (1978), Joyce et al. (1988), Richfield et al. (1991), Weeks et al. (1996)
DARPP32	Putamen↓ STN↓	Guo et al. (2012)
Enkephalin	Striatum↓ GPe↓	Emson et al. (1980), Deng et al. (2004), Tippett et al. (2007)
GAD	Striatum↓ GPe↓ GPi↓	Bird and Iversen (1974), Spokes (1980), Deng et al. (2004)
$GABA_A$ receptors	Striatum↓	Young et al. (1988), Faull et al. (1993)
$GABA_A$ receptor subunits		
$GABA_A$ α_1 subunit	GPe↑ GPi↑	Thompson-Vest et al. (2003), Allen et al. (2009)
$GABA_A$ α_3 subunits	GPe↑ GPi↑	Allen et al. (2009)
$GABA_A$ $\beta_{2,3}$ subunits	Striatum↓ GPe↑ GPi↑	Tippett et al. (2007), Allen et al. (2009)
$GABA_A$ γ_2 subunits	Striatum↓ GPe↑ GPi↑	Thompson-Vest et al. (2003), Allen et al. (2009)
$GABA_B$ receptor R1 subunit	GPe↑ GPi↑	Allen et al. (2009)
Glutamate		
Glutamate receptors	Striatum↓	Greenamyre et al. (1985)
GluA1 (AMPA)	Striatum↓	Dure et al. (1991)
GluN1(NMDA)	Striatum↓	Whitehouse et al. (1985), Young et al. (1988), Albin et al. (1990)
Neuropeptide Y	Striatum↑ (relative to volume)	Ferrante et al. (1987), Albin et al. (1990), Cicchetti and Parent (1996)
Parvalbumin	Striatum↓	Reiner et al. (2013)
Somatostatin	Striatum↑	Albin et al. (1990)
Substance P	Striatum↓ GPi↓ SNr↓	Marshall et al. (1983), Kowall et al. (1993)
Tyrosine hydroxylase	Striatum↑	Ferrante and Kowall (1987), Ferrante et al. (1987)

↓decreased expression ↑increased expression
The up arrows indicate increased protein detected and the down arrows indicate reduced protein detected mainly by immunohistochemical methodologies

were first identified using acetylcholinesterase (AChE) staining which was found mainly in the matrix by Graybiel and Ragsdale (1978). The smaller AChE-weak striosome compartment is identified by high concentrations of distinctive neurochemical markers such as neurotensin, LAMP, dopamine D2 receptors, $GABA_A$

receptors, substance P, and enkephalin while the larger matrix compartment is characterized by high concentrations of other neurochemicals AChE, tyrosine hydroxylase, somatostatin, the calcium-binding proteins CB, CR, PV, and the glutamatergic NMDA, and AMPA receptors (Faull and Villiger 1986; Voorn et al. 1989; Graybiel 1990; Dure et al. 1991; Waldvogel and Faull 1993; Manley et al. 1994; Holt et al. 1996, 1997; Parent et al. 1996; Prensa et al. 1999).

In HD, variable changes in the neurochemicals found in the striosome and the extrastriosomal matrix compartments have been reported. Some studies suggest that neuronal loss and gliosis shown by GFAP staining first appear in the striosomes (Hedreen and Folstein 1995), indicating that the neurons in striosomes may be more vulnerable at an early stage of HD or lower grades of the disease than those in the matrix (Morton et al. 1993; Hedreen and Folstein 1995; Augood et al. 1996). However, other studies show a preferential loss of neurons and neurochemical markers in the matrix compartment with clear sparing of the striosomes (Ferrante et al. 1987; Seto-Ohshima et al. 1988; Faull et al. 1993). These findings detailing the heterogeneous pattern of compartmental striatal degeneration in HD are interesting as studies in the rodent, and primate brains show that the striosome and matrix compartments have different patterns of connectivity and suggest that the two compartments are functionally different. Evidence from tracing studies suggests that the striosome compartment contains MSNs that receive inputs from the limbic system and these in turn project to the dopamine-containing neurons in the SNc (Gerfen 1984; Tokuno et al. 2002; Fujiyama et al. 2011). Therefore, the striosome compartment is thought to play a major "limbic" processing role in modulating mood and other related functions of the basal ganglia. In contrast, the matrix compartment receives topographically organized inputs from especially the sensorimotor and associative cortices, and hence, it is postulated to play a major role in the control of movement (Graybiel 1990; Parent et al. 1995; Parent and Hazrati 1995).

Extending the above observations, Tippett et al. (2007) have shown a differential pattern of degeneration in the two striatal compartments which correlates with the variable symptom profiles in 35 different HD cases (Fig. 6a–d). Some cases showed a selective striosomal loss of striatal neurons, enkephalin, and $GABA_A$ receptors, while others showed selective cellular and $GABA_A$ receptor loss in the matrix compartment. Other cases showed a mixed striosomal/matrix pattern of degeneration. Most importantly, this differential compartmental pattern of striatal degeneration between cases correlated in general principles with the variable symptom profiles between cases; most notably, cases with a profound degeneration in the striosomes correlated with major mood symptoms (Fig. 6c). By contrast, cases with marked degeneration primarily in the matrix compartment often had major motor symptoms (Fig. 6b). These findings suggest that the differential compartmental patterns of cell death and degeneration in the HD striatum could contribute significantly to the variability in HD symptomatology.

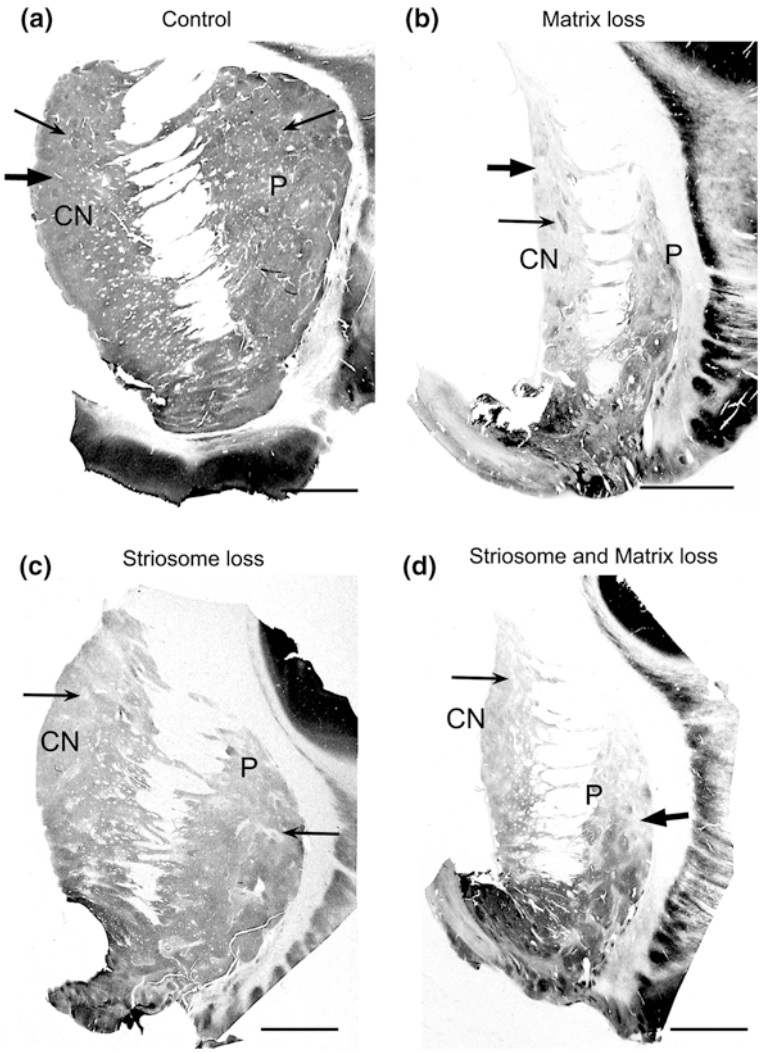


Fig. 6 Variability in the degeneration of the striosome and matrix compartments in different HD cases. Illustrations of the human caudate and putamen striatum stained for $GABA_A$ receptors which are highly expressed on projection neurons and interneurons in the human striatum. (**a**) Control human striatum, showing a relatively homogeneous staining pattern but with higher staining in the striosomes (*large arrows* indicate matrix, *small arrows* indicate striosomes). (**b**) Striatum from a Grade 3 Huntington's disease brain showing predominant matrix loss with preservation of striosomes. (**c**) a Grade 0 case showing predominantly striosome receptor loss with preservation of matrix, and (**d**) a Grade 3 case with a mixed loss of both striosome and matrix compartments. Modified from (Tippett et al. 2007). *CN* caudate nucleus, *P* putamen, Scale bar = 5 mm

5.3 Globus Pallidus

The globus pallidus shows atrophy and cell loss of approximately 40 % in Grades 3 and 4 with the external segment much more involved than the internal segment. In Grade 4, the GP shows 50 % volume loss (Lange et al. 1976). Using specialized MRI techniques, globus pallidus volume changes of approximately 50 % were detected (Douaud et al. 2006). The external segment is more involved than the internal segment, and atrophy of neurons and reactive astrocytes are discrete especially in Grade 4. These changes are almost constantly observed in Grade 4 brains from cases with juvenile onset (JOHD) of symptoms, or with the rigid-akinetic rather than the choreic form. According to Lange et al. (1976), the absolute number of pallidal neurons decreases up to 40 %, but the neuronal density is up to 42 % higher than in control GPe and 27 % higher in the GPi, reflecting the major atrophy of the globus pallidus in HD. The neurons are smaller and more densely packed than normal in Grade 3, and even more so in Grade 4, suggesting that the onset of the loss of the neuropil might occur before that of the neurons. (Spielmeyer 1926; Schroeder 1931; Neustaedter 1933; Campbell et al. 1961; McCaughey 1961; Vonsattel et al. 1985).

There is also a very marked loss of cannabinoid CB1 receptors in all of the striatal output nuclei (GPe, GPi, and SNr; Fig. 5), which is evident even at Grade 0 where there is minimal cell loss in the striatum (Glass et al. 1993, 2000; Richfield and Herkenham 1994; Allen et al. 2009). This may indicate dysfunction of the cannabinoid system in basal ganglia pathways in the very early stages of HD. On the other hand, associated with the loss of neurotransmitter GABA from the striatopallidal and striatonigral pathways, there is a major increase in postsynaptic $GABA_A$ (Fig. 3g, h) and $GABA_B$ receptors, which is proposed to be a compensatory upregulatory response of GABA receptors on the pallidal and nigral output neurons (Figs. 3g, h, and 5) (Penney and Young 1982; Faull et al. 1993; Allen et al. 2009).

5.4 Substantia Nigra

There is a loss of neurons in the SNr (Lewy 1923; Spielmeyer 1926; Schroeder 1931; Hallervorden 1957; Campbell et al. 1961; Richardson 1990). The SNc is thinner than controls, yet its number of neurons were originally reported to be apparently normal in all grades giving the impression of an increased density of pigmented neurons (Campbell et al. 1961; Richardson 1990). However, other studies on the SNc have found cell loss (Oyanagi and Ikuta 1987; Oyanagi et al. 1989) but less than that of the SNr (Ferrante et al. 1989). In addition, a loss of TH protein and mRNA from the SNc, and loss of TH in the matrix of the striatum has been reported (Ferrante and Kowall 1987). Neurons of the SNc have a major dopaminergic projection to the full extent of the striatal matrix, and these studies suggest that a loss of dopamine in the striatum may contribute to the symptoms of HD (Yohrling et al. 2003).

5.5 Subthalamic Nucleus

In the STN, there is a discrepancy between the marked atrophy present in Grades 3 and 4 (up to 25 % volumetric loss) and the scarcity of the reactive astrocytes (Spielmeyer 1926; Lange et al. 1976). A recent study has measured the loss of neurons in the STN to be on average 20 % less than controls (Guo et al. 2012) although this did not always correlate with cell loss in the putamen, suggesting the STN cell loss trails that of the putamen. Whether the changes involving these structures are due to the mutation alone or secondary to the preponderant involvement of the striatum, or a combination of both remain to be determined. It would be interesting to know to what extent the loss of neurons in the cerebral cortex which form the hyperdirect pathway to the STN would reduce the excitation of the STN in addition to the decreased inhibition of the STN through changes in the indirect pathway due to the loss of striatal neurons in the disease process.

5.6 Cerebral Cortex

Cortical degeneration in HD has been observed and reported over many years and has recently been examined in more detail with the advent of modern imaging techniques and stereological counting techniques.

Cortical atrophy has been observed in HD brains, especially in those in advanced stages of the disease. Many studies of the cortex in HD brains have found evidence of overall cortical volume loss, cortical thinning, and neuronal loss (de la Monte et al. 1988; Cudkowicz and Kowall 1990; Hedreen et al. 1991; Macdonald et al. 1997; Rajkowska et al. 1998; Macdonald and Halliday 2002; Rosas et al. 2002, 2008; Ruocco et al. 2008; Thu et al. 2010).

The earliest accounts of cortical neuropathological features were described by several authors including Bryun (1968), Forno and Jose (1973), Tellez-Nagel et al. (1974), Lange et al. (1976), Hadzi et al. (2012) and Trifiletti et al. (1987). Evidence of global cortical atrophy has been observed by de la Monte et al. (1988) where the authors demonstrated overall morphometric atrophic changes in the brain (30 % of mean brain weight reduction) with 21–29 % reduction in the gray matter and 29–34 % loss in the white matter, and Halliday et al. (1998) showed that the degree of cortical volume loss was similar in the frontal, temporal, parietal, and occipital lobes (19 % reduction of total brain volume) with no major change in volume in the medial temporal lobe. The amount of cortical volume loss was demonstrated to correlate with the degree of striatal atrophy and the number of CAG repeats, suggesting that the disease processes in the striatum and cortex are related. A significant reduction in the frontal lobe volume (17 %) and frontal white matter volume (28 %) was also found (Aylward et al. 1998).

Several detailed investigations of cellular changes in the cerebral cortex in HD brains have shown that neuronal cell body size and cell number are decreased in the

HD cortex (Macdonald et al. 1997; Rajkowska et al. 1998; Macdonald and Halliday 2002), and in some studies, this neuronal loss has been shown to be layer specific (Hedreen et al. 1991; Sotrel et al. 1991, 1993). Laminar-specific neuronal degeneration was found in the HD cortex in 11 HD cases where there was a significant loss of pyramidal projection neurons in layers III and V in the superior frontal cortex and cingulate gyrus (Cudkowicz and Kowall 1990). A study by Hedreen et al. (1991) of five postmortem HD brains showed that significant neuronal loss was present in layers V and VI of prefrontal cortex in Grade 4 HD brains. Layer VI was found to demonstrate the greatest loss in thickness, while layers III and V were also atrophied in HD.

Since neurons from layers III and V project mainly to the striatum, it has been suggested that cortical cell loss in HD is a result of retrograde degeneration secondary to striatal pathology. However, the study by Hedreen et al. (1991) showed that extensive degeneration of layer VI was also present in the cerebral cortex of early-stage HD brains. As neurons in layer VI have major local, subcortical, intracortical projections as well as projections to the thalamus, the claustrum, and other regions of the cortex, this study suggested that cortical cell loss in HD is a disease process parallel to striatal degeneration and not a secondary process as was originally believed (Cudkowicz and Kowall 1990; Hedreen et al. 1991; Sapp et al. 1999). Sotrel et al. (1991) also investigated neuronal degeneration in cortical neurons in the dorsolateral prefrontal cortex in HD. Loss of specific subpopulations of large pyramidal neurons in layers III, V, and VI was demonstrated in an investigation of 81 HD brains, as well as a decrease in the thickness of the cortical layers containing the cell bodies of these neurons with shrunken dendritic trees and sparse spines in advanced stages (Sotrel et al. 1991, 1993; Selemon et al. 2004).

Another study by Rajkowska et al. (1998) investigated neuronal degeneration in the prefrontal cortex in seven HD cases, by measurements of the size and density of both neurons and glial cells in the HD tissue compared to the control tissue. In HD cases, the neuronal size and density of large neurons in the prefrontal cortex was reduced by 9 %; this change was pronounced in pyramidal cell layers III, V, and VI, with no significant decrease in mean cell body size in layer II and VI neurons, and in layer VI, the decrease in size of large neurons was accompanied by a relative increase in the size of small neurons. The density of large glial cells was greatly increased in all layers of the HD prefrontal cortex. As the large neurons showing reduced size and density in the prefrontal cortex in HD are primarily in layers III, V and VI, these cells may be large projection neurons that form corticocortical, corticostriatal, and corticothalamic projections. These studies have focused mainly on the prefrontal cortex, as its role in behavior suggests that neural changes in the prefrontal cortex may contribute to the behavioral aspects of HD (Watkins et al. 2000).

A more detailed quantitative study using stereological cell counting has been addressed by Heinsen et al. (1994), and the authors found a pronounced pyramidal cell loss in the supragranular layers in the primary sensory areas including primary somatosensory cortex (areas 3, 1, 2), primary visual cortex (area 17), primary auditory cortex (area 41), and association areas of the frontal, parietal, and temporal

lobes. Similarly, Macdonald et al. (1997) reported a significant reduction of pyramidal cells across layers III and V, and also found atrophy of cell bodies of the remaining cells in the angular gyrus of the parietal lobe. In the following studies, Macdonald and Halliday (2002) investigated cellular changes in the motor cortical regions, i.e., primary motor cortex (area 4), supplementary and premotor region (area 6), and cingulate motor cortex (posterior part of area 24), and observed a significant reduction of total neuronal number in the primary motor cortex (42 % loss) and the premotor region (49 % loss) in HD. No significant change was observed in the posterior cingulate motor region. In addition, there was a significant loss of pyramidal cells (41 % loss) in the primary motor cortex in HD. Pyramidal cells in the primary motor cortex are involved in the corticostriatal pathways, with the putamen receiving many inputs from the primary and association motor areas. In addition, it has been shown that isolated lesions of the putamen in humans cause chorea in only a few cases. This suggests that corticostriatal degeneration may be important in the development of chorea in HD cases (Bhatia and Marsden 1994; Macdonald and Halliday 2002).

The neuropathological changes in HD have been shown to vary in different areas of the cortex in different stages of the disease, suggesting that they may be related to the symptoms of HD such as motor abnormalities, dementia, apathy, irritability, mood, depression, and visual disturbances (Hedreen et al. 1991; Halliday et al. 1998; Rosas et al. 2002, 2008; Tippett et al. 2007; Thu et al. 2010). More recently, the advances in detailed structural neuroimaging methods have facilitated important steps in elucidating the cortical basis of the clinical heterogeneity in HD patients (Montoya et al. 2006), for example, several authors have demonstrated utilizing in vivo MRI evidence for regional and progressive thinning of the cortical gray matter in both symptomatic and premanifest HD cases which correlates with the clinical expression of the disease (Jernigan et al. 1991; Rosas et al. 2002, 2005, 2008; Kassubek et al. 2004; Douaud et al. 2006; Nopoulos et al. 2007, 2010; Paulsen 2009; Tabrizi et al. 2011). Importantly, Rosas et al. (2002) showed widespread cortical thinning of 11 HD cases with varying clinical severity. The thinning of the cortex appeared to be progressive and followed a posterior to anterior regional pattern of cortical degeneration. Cortical thinning also occurred early in the disease and showed specific regional thinning in different cases. The greatest amount of thinning was observed in the sensorimotor cortex in cases at all stages of the disease. In addition, the primary motor (area 4), sensory (superior portions of areas 3, 2, 1) and visual cortical regions were the most affected, and the thinning was extended to other regions that include posterior superior frontal, posterior middle frontal, superior parietal, and the parahippocampal gyrus (Rosas et al. 2008). The degree of thinning varied between different cortical regions, with the greatest thinning of more than 15 % occurring in the primary visual and primary motor cortices. In addition, the decrease in cortical thickness was found to progress from sensorimotor and primary visual cortical areas to include frontal motor association cortex, parieto-occipital cortex, entorhinal cortex, and eventually the entire cortex. Furthermore, the thinning in the different cortical areas correlated with the varying cognitive deficits and motor disorder of the different individuals with

HD. Also, anterior cingulate cortex atrophy has been found to correlate with emotion and depression clinical scores in HD cases (Hobbs et al. 2011).

In line with the in vivo imaging investigations, in a recent pathologic study, the variable neuropathology in the cerebral cortex has been correlated with specific symptoms of HD (Thu et al. 2010) (Fig. 7). Detailed stereological cell counts in motor and cingulate cortical regions of 12 HD cases have shown a variation in the total number of neurons (NeuN) in the primary motor cortex (24 % loss), and anterior cingulate cortex (36 % loss). In addition, the number of SMI32-positive pyramidal neurons was also affected in these regions which followed the pattern of total neuronal loss, with 27–34 % reduction in the pyramidal cell number in the two cortical regions. Interestingly, the loss of total neurons and pyramidal neurons varied between HD cases which expressed different clinical symptoms (Thu et al. 2010). For example, a significant cell loss in the primary motor cortex was associated with HD cases with predominant motor abnormalities (28 % loss in total neuronal population Fig. 7a, b) but no significant cell loss was observed in the motor cortex in HD cases with major mood symptoms (Fig. 7c). In contrast, a significant cell loss in the anterior cingulate cortex was associated with HD cases with dominant mood symptom profiles (54 % loss in total neuronal population Fig. 7f), but no significant loss was observed in the cingulate cortex in HD cases with a dominant motor symptom profile (Fig. 7e). This study clearly illustrated for the first time how cortical degeneration in specific functional brain regions correlates with varying symptom profiles of different HD cases.

Although degeneration of cortical pyramidal neurons in layers III, V, and VI has been well documented, relatively few studies have been conducted in the HD cortex on cortical interneurons. Pathological studies on the cortical interneurons have shown that there was relative sparing of PV and neuropeptide Y (NPY) expressing interneurons in the superior frontal cortex of HD cases (Cudkowicz and Kowall 1990) and Macdonald and Halliday (2002) showed no significant change in the interneuron populations defined by CB, CR, and PV in motor cortical regions in five HD cases examined in their study. In contrast, Ferrer et al. (1994) observed a significant decrease in PV expressing interneurons in the frontal cortex, but significant difference was not observed in the occipital and temporal lobes. These results suggest that there is a heterogeneous topographical pattern of GABAergic interneuron loss in the different functional regions of the cortex in HD.

Extending these observations, our recent preliminary studies on the pattern of cell loss of cortical interneurons in the motor and cingulate cortex demonstrated a heterogeneous loss of interneurons in the two cortical regions in HD cases with different symptom profiles compared to control cases. These findings suggest that the loss of inhibition of pyramidal cells by the death of specific types of interneurons in HD may be a critical determinant in shaping the output activity of the cerebral cortex. The differential loss of inhibition by these interneurons in different cortical regions may lead to hyperexcitability of the pyramidal neurons which may further exacerbate the disease process and contribute to the striatal excitotoxic processes in HD (Beal 1994; Sieradzan and Mann 2001; Cepeda et al. 2007).

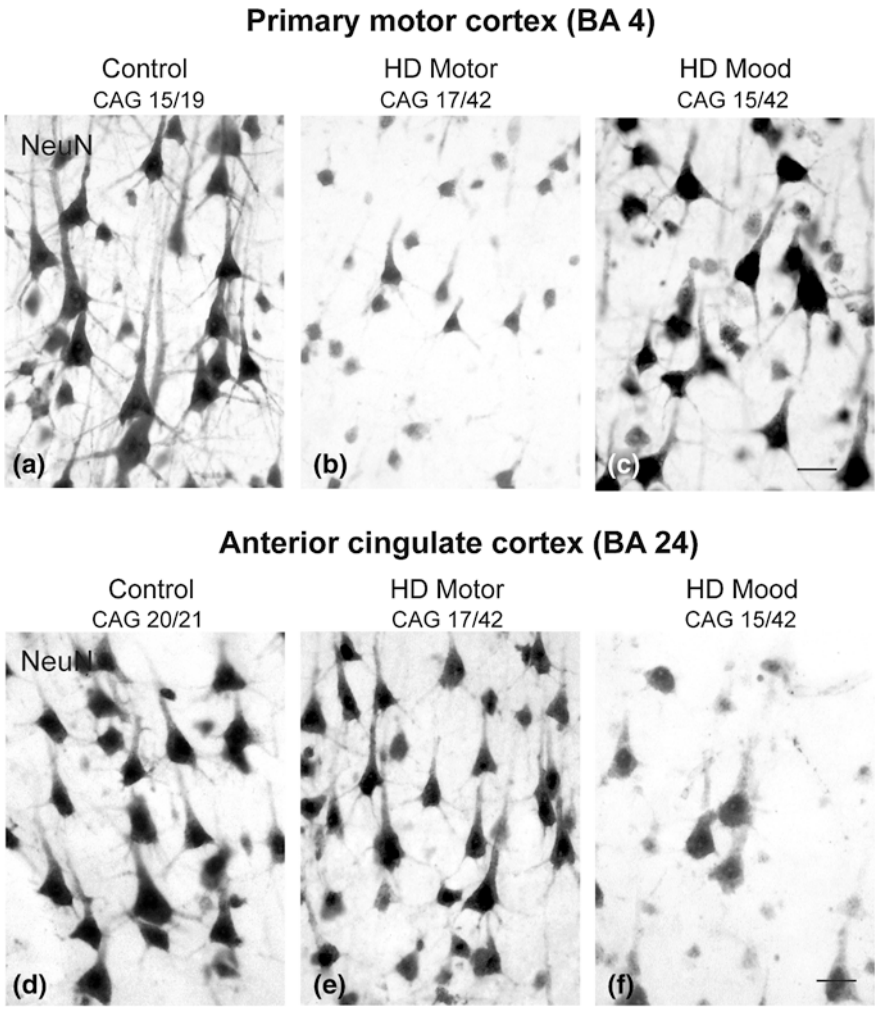


Fig. 7 Cell loss in the primary motor cortex and cingulate cortex in Huntington's disease. **a–c** Illustrates variability in the labeling of cortical neurons stained with neuronal marker Neuronal (NeuN) in the primary motor cortex, showing marked cell loss in the HD motor cortex in *motor* dominant cases (**b**), but no loss in *mood* dominated cases (**c**). **d–f** Variability in the labeling of cortical neurons stained with neuronal marker Neuronal (*NeuN*) in the anterior cingulate cortex showing marked cell loss in the HD cingulate cortex in *mood* dominated cases (**f**), but no loss in *motor* dominated cases (**e**). Modified from (Thu et al. 2010). Scale bars **a–c**, **d–f** = 30 μm

6 Other Brain Regions

In general, the grade of striatal degeneration correlates with the atrophy of other brain regions than the striatum in HD. In general, in Grades 1 and 2, non-striatal structures of the brain are apparently normal, or show only mild atrophy, unless

there is age-related volumetric loss, or a superimposed disease, such as Alzheimer disease. However, in Grades 3 and 4, non-neostriatal structures including globus pallidus, neocortex, thalamus, STN, substantia nigra, white matter, and cerebellum are smaller than normally expected. As detailed below, these gray matter structures may show mild or marked neuronal loss usually without reactive astrocytosis, the most frequent exceptions being the GPe and the centromedian nucleus of the thalamus especially in Grade 4, and to a lesser extent in Grade 3.

6.1 Thalamus

The thalamus is often within normal limits on gross examination. The centrum medianum shows astrocytosis and neuronal loss especially in Grade 4, and to a lesser extent, in Grade 3; otherwise the thalamus is microscopically normal in lower grades. A recent MRI analysis (Kassubek et al. 2005) shows that there is variability in thalamic degeneration which agrees with neuropathological studies in the post-mortem brain. The main regions of atrophy described in the thalamus so far are that of the dorsomedial nucleus (DM) (Heinsen et al. 1999), the centromedial/ventro-lateral nucleus (CM/VLa) nuclear group, and the centromedial/parafascicular nucleus (Heinsen et al. 1996). The parafascicular nucleus is part of the thalamic intralaminar nuclear group which projects to the striatum and its terminals are thought to label specifically for VGlut2 based on animal studies (Doig et al. 2010; Deng et al. 2013). The loss of these terminals is thought to occur in HD, but this is still not confirmed in human HD striatum.

6.2 Hypothalamus

The hypothalamus contains a large number of interconnected nuclei involved in regulation of metabolic functions as well as the control of sleep. Sleep disturbances, alterations in circadian rhythm, and weight loss have been found to be altered in HD patients (Morton et al. 2005; Petersen et al. 2005; Petersen and Bjorkqvist 2006; Aziz et al. 2008; Hult et al. 2010). Atrophy of the lateral tuberal nucleus (LTN) in the basolateral region of the hypothalamus in HD cases was described by Vogt and Vogt (1951). Further neuropathological changes were described by Kremer et al. (1990, 1991) and Kremer (1992) with up to 90 % neuronal cell loss as well as gliosis in the LTN of the hypothalamus. Other studies in the lateral hypothalamus have found a loss of orexin (hypocretin)-positive and somatostatin co-expressing neurons in the in HD cases (Timmers et al. 1996; Petersen et al. 2005; Aziz et al. 2008). Also gray matter atrophy in the hypothalamus using voxel-based MRI analyses (Kassubek et al. 2004; Douaud et al. 2006) and in vivo PET studies (Politis et al. 2008) have been observed in early stage and symptomatic HD cases. However, the detailed

neuropathology of hypothalamic cell populations in the various hypothalamic nuclei in HD and their role in the overall pathogenetic mechanisms are yet to be determined.

6.3 Hippocampus

Reports on the hippocampal involvement in HD have been outlined in several studies. An early study showed no reduction in cell density in the hippocampus in HD (Dunlap 1927) however a more recent morphometric study has reported a reduction of hippocampal area of about 20 % in 30 HD patients (de la Monte et al. 1988). Also significant volume reductions in the hippocampal region (9 % volume reduction compared to control volume) have been observed early in patients with HD (Rosas et al. 2003). Additionally, Vonsattel and DiFiglia (1998) observed neuronal loss and reactive gliosis in the hippocampal formation in a number of HD cases. A further quantitative stereological technique to assess neuronal populations in four areas of the hippocampus (the granule cell layer of the dentate and CA1, CA3, and CA4 fields) in 11 HD cases showed significant changes in neuronal density restricted to the CA1 region while no significant decrease was observed in the other regions of the hippocampus (Spargo et al. 1993). Selective vulnerability of various regions of the hippocampus in HD is yet to be fully determined, and whether the variation reported in studies to date reflects a variation in cases with different symptom profiles.

6.4 Cerebellum

The neuropathological findings pertaining to the cerebellum in HD were for a longtime controversial, perhaps because they were mainly obtained using conventional methods of neuropathological evaluation. However, Rüb et al. (2013) recently found that the HD mutation is specifically harmful to neurons of the cortical and deep nuclei of the cerebellum. The conflicting findings regarding the HD cerebellum are multifactorial including the use of a wide range of methods applied for the analyses. For example, Dunlap reported that among the 29 cases with chronic chorea (17 with proven family history), only one case with HD had cerebellar atrophy. He identified the fraction of the weight of cerebrum/cerebellum to be 1/5.8 in HD compared to 1/7.2 in controls (Dunlap 1927). Spielmeyer described gliosis involving gray and white matter without systematic selectivity in the cerebella of two cases (Spielmeyer 1926). McCaughey found “possible patchy loss of Purkinje’s cells” in six, and loss of neurons involving the dentate nucleus in nine of his series of 21 HD brains (McCaughey 1961). Rodda found three in “about 300” HD brains, which showed “severe atrophy of the cerebellum” (Rodda 1981). One of those three cases had adult onset symptoms, and no definite family history

of HD. The third patient had a family history of HD, epilepsy, and died at the age of 6 years. Jeste et al. (1984) conducted a quantitative study of the cerebellar cortex of 17 HD cases, two of whom had epilepsy. There was no cerebellar atrophy noticed on gross examination. They found a decrease (up to 50 %) of the density of Purkinje cells but normal thickness of granular and molecular layers. The Purkinje cell loss was variable in its extent in different cases.

Cerebellar atrophy is often reported in cases with JOHD (age of onset <20 year) (Harper et al. 1991). The four cases with JOHD and severe cerebellar atrophy reported by Jervis all had epilepsy (Jervis 1963). The nine-year-old patient reported by Markham and Knox had epilepsy, severe cerebellar atrophy, but "no focal atrophy in Sommer's sector" (Markham and Knox 1965). Byers et al. reported four juvenile HD cases all with severe cerebellar atrophy (Byers et al. 1973). The hippocampal formation was available in three of the four cases; of these three hippocampi, two showed neuronal loss, and reactive gliosis suggesting that to some extent the cerebellar atrophy may have been secondary to remote hypoxic-ischemic events. Juvenile HD cases are prone to seizures. Thus, seizures may account for some cerebellar or hippocampal neuronal loss, two sites notably vulnerable to hypoxic-ischemic events.

By conventional methods of evaluation, the cerebellum is smaller than normally expected in Grades 3 or 4. Despite this volume loss, neuronal density in the cerebellar cortex frequently appears within normal limits. Segmental loss of Purkinje cells with or without Bergmann gliosis may occur; however, these changes are inconsistent. As mentioned, Rüb et al. (2013) have shown recently with quantitative studies that the cerebellum is a site of primary degeneration in HD and recently were able to find that the HD mutation is specifically harmful to neurons of the cerebellar cortex and all of the deep nuclei of the cerebellum. These investigators compared eight, well-characterized HD cerebella with eight control cerebella using morphometric analysis and immunohistochemistry and found a pronounced loss of neurons especially in the fastigial nucleus as well as loss of CB-labeled Purkinje cells throughout the cerebellum. The Purkinje cells also showed disrupted dendrites and cytoplasmic inclusions.

6.5 *Subventricular Zone and Neurogenesis in Huntington's Disease*

The subventricular zone (SVZ) which lies along the margin of the caudate nucleus adjacent to the lateral ventricle has become a region of intense interest with the discovery of adult neural stem cells in this region. In the control, human SVZ neural precursors have been identified (Curtis et al. 2005; Kam et al. 2009) using PCNA as a marker for proliferating cells coupled with neuronal stem cell markers to identify their neuronal phenotype. In HD, an increase in cell proliferation was found in the SVZ with evidence for increased neurogenesis and increasing thickness of the SVZ

with increasing grade (Curtis et al. 2003). This raises the exciting possibility of stimulating the production of new neurons through neurogenesis from precursors in the SVZ and subsequent migration directly or via the rostral migratory stream (Curtis et al. 2007) into the cell-depleted HD striatum as a possible therapy for HD. The subsequent integration of these newly formed neurons into the basal ganglia circuitry will be critical. Studies in the rat indicate that stem cells have the ability to migrate into the quinolinic acid lesioned rat striatum (Tattersfield et al. 2004) but further studies are needed to determine whether this is a potentially viable therapy in humans. New research into reprogramming fibroblasts or other cell types to produce new neurons is ongoing (Vierbuchen et al. 2010), with the possibility of transplanting new neurons into regions of neuronal death as a form of treatment for HD.

The other neurogenic region in the human brain is the subgranular zone of the dentate gyrus in the hippocampus where neurogenesis in the human brain was first shown (Eriksson et al. 1998). Intensive research has followed this discovery especially in animals as to what drives this neurogenesis. However, a recent study in the human brain has found no increased neurogenesis in this proliferative zone in the Huntington's diseased brain (Low et al. 2011). This in contrast to the SVZ in the same brains and agrees with animal models of HD, which also tend to show no proliferation in the hippocampus (Curtis et al. 2012).

7 Gliosis

Reactive gliosis is defined as the increase in number and activation of astrocytes, microglia, and oligodendrocytes and form part of the inflammatory response of the diseased brain. Increases of the three types of glial cells astrocytes, microglia, and oligodendrocytes have been observed in the Huntington's diseased brain in a range of observations starting from the earliest historical studies (Roizin et al. 1976). These studies found a heterogeneous pattern of gliosis throughout the brain. More recent studies found increased gliosis occurred in the dorsal striatum, which was the region of major cell loss in HD (Myers et al. 1991). Additionally, in the caudate nucleus, microglia and astrocytes increased with increasing grade particularly near the ventricular edge and internal capsule, while oligodendrocytes were markedly increased in all grades and were localized throughout the degenerated region of the caudate nucleus. Focal regions of astrocytic gliosis within the striatum that were identified as striosomes were found in lower grades of HD (Hedreen and Folstein 1995), and this finding proposed that the earliest changes in HD were associated with striatal striosomes. More specific markers for microglia found that activated microglia correlated with neuronal loss in the neostriatum, globus pallidus, cerebral cortex, and white matter, but microglia displayed a different morphology in the striatum compared to the cerebral cortex. In the cerebral cortex, they were associated with the dendrites of pyramidal cells (Sapp et al. 2001). Microglia also stain with ferritin, an iron storage protein, and activated microglia were shown to contain abnormally high levels of iron in HD (Simmons et al. 2007). Newer methods using

positive emission topography have investigated microglial activation in the brain of HD and have found widespread microglial activation throughout the striatum, pallidum, frontal and cingulate cortices and brainstem in HD cases, which was evident also in presymptomatic *HTT* gene carriers (Gomez-Tortosa et al. 2000; Pavese et al. 2006; Tai et al. 2007). This close association of neuronal dysfunction and microglial activation needs further investigation. Astrocytes in the white matter of the HD brain were shown to have intranuclear inclusions (Shin et al. 2005), and it was estimated that approximately 12 % of astrocytes have nuclear inclusions in late-stage HD. The metabolism of astrocytes may thus be compromised and therefore may play a role in metabolic dysfunctions of the HD brain.

8 Aggregates

The wild-type huntingtin is a large protein (3144 amino acid residues) expressed mostly in the cytoplasm, dendrites, and axon terminals of neurons in the brain (Trottier et al. 1995; Ferrante et al. 1997). Huntingtin is associated with various intracellular organelles, including endoplasmic reticulum (ER), Golgi apparatus (DiFiglia et al. 1995; Hilditch-Maguire et al. 2000), and microtubules (Li et al. 2003). A small proportion is also found in the nucleus (Kegel et al. 2002). Huntingtin has no clear homology to known proteins, and its normal function is yet to remain fully understood. However, a considerable effort in understanding huntingtin structure and function has led to suggested roles in development (Duyao et al. 1995; Zeitlin et al. 1995), protein trafficking (Huang et al. 2004; Li and Li 2004), anti-apoptotic role (Zeitlin et al. 1995; Lunkes et al. 2002), and transcriptional regulation (Zuccato et al. 2003). The mutant huntingtin (mHtt) shows a similar expression level and regional distribution to the wild-type huntingtin in the brain (Aronin et al. 1995), but a difference in huntingtin epitope localization has been observed, and abnormal accumulation of *N*-terminal fragments of mutant huntingtin form aggregates/inclusions in the nucleus, cytoplasm, and dystrophic neurites in HD brains (Davies et al. 1997; DiFiglia et al. 1997). These protein aggregates are thought to be formed by associations of polyglutamine (polyQ) regions, which act as a "polar zipper" (Perutz et al. 1994; Perutz 1996). Protein aggregates have been observed in immunohistochemical studies using various antibodies directed against the huntingtin *N*-terminal region such as EM48 (Gutekunst et al. 1999; Hodgson et al. 1999), S830 (Landles et al. 2010), huntingtin protein (Goldberg et al. 1996; Zuccato et al. 2001), and antibody 1C2, which is directed against the CAG repeat region of the TATA-binding protein (Trottier et al. 1995; Herndon et al. 2009) (Fig. 8). It has been suggested that the toxic influence of these inclusions leads to a differential loss of specific subsets of neurons; however, this is still a topic of debate as there is evidence for both deleterious and protective effects of huntingtin aggregation (Davies et al. 1999; Arrasate et al. 2004; Reiner et al. 2007). The important steps of the aggregate toxicity hypothesis may involve proteolysis, nuclear translocation, and aggregation. The mHtt possess a higher

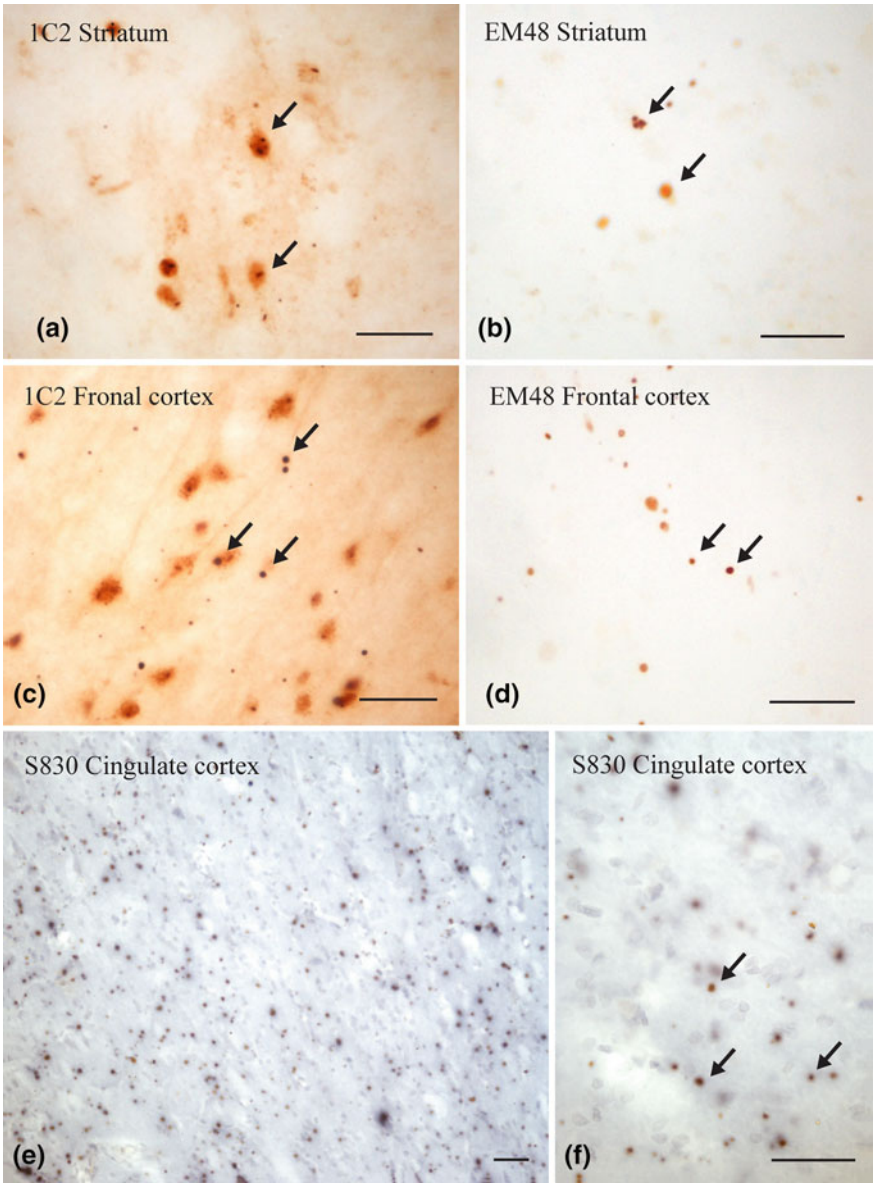


Fig. 8 Aggregates in the human cerebral cortex and striatum in Huntington's disease. Photomicrographs showing various types of aggregate labeling in the human brain in Huntington's disease cases localized with different antibodies directed against the *N*-terminal region of the huntingtin protein (Htt) and the CAG repeat region of the TATA-binding protein. **a**, **b** Aggregates labeled with (**a**) 1C2 (raised against the expanded CAG repeat in the TATA-binding protein) and (**b**) EM48 (raised against amino acids 1–256 of Htt) (*arrows*) in the striatum of HD brain. **c**, **d** Aggregates labeled with (**a**) 1C2, (**b**) EM48 shown by *arrows* in sections of the frontal cortex of HD brain. **e**, **f** S830 directed against the *N*-terminus of human huntingtin exon 1 protein stained in the cingulate gyrus of HD brain (**e**) at low magnification and (**f**) at high magnification. illustrating immunodetection of polyQ aggregates (indicated by *arrows*). Scale bars **a**, **b**, **d**, **f** = 40 μm; **c**, **e** = 50 μm

likelihood of proteolytic cleavage than its wild-type counterpart (Goldberg et al. 1996; Saudou et al. 1998). The smaller cleaved fragments are suggested to be more toxic (Gong et al. 2008), and the toxicity is also associated with nuclear translocation (Martindale et al. 1998; Atwal et al. 2007).

The immunohistochemical analyses of postmortem human HD brain (Fig. 8) have demonstrated the presence of aggregates that can form neuronal intranuclear inclusions (NIIs) or cytoplasmic and neuropil extranuclear inclusions (NEIs), and huntingtin aggregates also form in axons and dendritic spines. The nuclear aggregates were originally mentioned as "filamentous inclusions" examined using electron microscopy in the neuronal nuclei of the human HD brain (Roizin et al. 1979). Thus, aggregates are described as being made of granular and filamentous material, which is not membrane bound. NIIs tend to be round, oval, or rod shaped, larger than the nucleolus and more frequent in juvenile than adult onset cases. In contrast, the neuropil aggregates occur more frequently than NIIs in adult cases and tend to be round or oval and may be arranged in thin extensions along a process (DiFiglia et al. 1995, 1997; Gutekunst et al. 1999; Maat-Schieman et al. 1999) (see Fig. 8). The inclusions are present prior to symptomatic development of the disease in the HD human brain and found throughout the cortex, but less frequently in the striatum (Becher et al. 1998; Gutekunst et al. 1999; Maat-Schieman et al. 1999; Van Roon-Mom et al. 2006; Herndon et al. 2009). Within the cortex, the cells tend to display combinations of nuclear and cytoplasmic as well as neuropil aggregations (Herndon et al. 2009) with the highest levels of intranuclear inclusions found in juvenile cases, which tend to have relatively very high CAG repeat numbers (Gutekunst et al. 1999). Elucidating the exact molecular mechanisms for mHtt cytotoxicity is an ongoing challenge. The mHtt aggregates are mostly ubiquitinated and are enriched in truncated polyglutamine containing fragments generated by several proteases; however, the precise mechanisms responsible for the toxicity of these proteolytic products remain elusive. It is generally thought that mHtt confers a toxic gain-of-function, which elicits a cytotoxic cascade. Indeed, overexpression in various types of cells is cytotoxic (Lievens et al. 2008; Weiss et al. 2009). However, the soluble, non-aggregated forms of mHtt in tissues have been implicated more recently to be the neurotoxic culprit (Saudou et al. 1998; Arrasate et al. 2004; Kitamura et al. 2006; Ratovitski et al. 2009). By contrast, there is also evidence to suggest that the aggregated forms of mHtt may have no effect or even be protective to cells (White et al. 1997; Saudou et al. 1998; Arrasate et al. 2004). The substances that are toxic to cells generally elicit a myriad of effects, and therefore, it is difficult to isolate which are primary, secondary, or tertiary (Landles and Bates 2004; Ross and Poirier 2004; Kaltenbach et al. 2007). In addition, the expression of huntingtin does not reflect the distribution of selective vulnerability (Kuemmerle et al. 1999). Some have even presented the view that cortical neurons may actively destroy MSNs in the striatum, rather than the MSN death being due to specifically mHtt itself (Fusco et al. 1999). Nevertheless, inclusions play a role in HD and are commonly used as biological markers for the testing and development of new therapeutic strategies aimed at reducing inclusion formation (Yamamoto et al. 2000; Schiefer et al. 2002; Rodriguez-Lebron et al. 2005; Machida et al. 2006).

9 White Matter Changes

White matter changes have been reported in HD brains, although detailed systematic studies have not been carried out. Earlier studies such as de la Monte et al. (1988) measured 29–34 % changes in overall white matter area in slices through HD brains although these varied with the level measured. The loss of white matter correlated closely with the amount of cerebral cortex gray matter lost, and in addition, the loss of white matter and cerebral cortex correlated more strongly with dementia and depression (de la Monte 1988). Activated microglia and activated astrocytes have been variably reported in the white matter tracts (Vonsattel et al. 2011), but detailed studies of gliosis throughout the white matter have not been reported. Most of the more recent studies on white matter changes in HD have been carried out with neuroimaging techniques such as DTI and MRI. These have shown pre-symptomatic changes in the white matter in the brain throughout different regions. In particular, specific changes are detected in the microstructure of the corpus callosum as well as the internal capsule more than a decade before onset of symptoms which may reflect degeneration of cortical pyramidal neurons, loss of cortical connectivity, and compromised associative processing leading to cognitive deficits in HD (Rosas et al. 2006, 2010). Also disproportionate loss of white matter was found in the prefrontal cortex (Aylward et al. 1998) in HD brains.

10 Degeneration in Peripheral Tissues

Although the most dramatic changes in HD occur in the brain, abnormalities of peripheral tissues have also been recently documented. Non-neurological abnormalities of HD include weight loss, muscle wasting, cardiac problems, insulin sensitivity, and gastrointestinal disorders, and many of these may be due to malfunctions in the peripheral tissues. These are only recently being recognized and documented in studies in humans and in animal models, and is highlighted in a recent review by Van der Burg et al. (2009).

The following peripheral tissues are affected in the disease. The digestive system is known to be affected which may affect nutrient uptake. Patients suffer from xerostomia or dry mouth, which may be due to lack of saliva, and also can affect taste and swallowing, which patients also suffer from. Ghrelin-producing cells, which produce Grehlin that aids in food intake, are reduced in number in the stomach. The pancreas is affected, and the islet cells are atrophic and contain nuclear inclusions, which may lead to the high incidence of impaired glucose tolerance and diabetes (Podolsky et al. 1972; Andreassen et al. 2002; Hunt and Morton 2005; Lalic et al. 2008). Another major problem in HD patients is skeletal muscle wasting despite being highly active due to hyperkinesia and chorea characteristic of HD, and in HD mice, there are aggregates in the muscle cells (Ribchester et al. 2004); additionally, there are also mitochondrial enzyme dysfunctions in the muscles of HD patients (Arenas et al. 1998).

Cardiac failure is much higher in the HD population with a 30 % incidence compared to 2 % in the normal population and a leading cause of death in HD (Lanska et al. 1988). It is unclear whether this is from the underlying genetic effects on the heart muscle or due to peripheral nerve damage.

The testes are also affected, and this correlates with the very high levels of Htt protein found in testicular tissue. There is testicular degeneration (Van Raamsdonk et al. 2007) with degeneration of the germ cells and thickening of the seminiferous tubes, which appeared to correlate with CAG repeat length.

All of these studies indicate that it is not just the brain that is affected although it is still not clear if these effects are directly gene related or related to endocrine imbalance from the hypothalamus, from peripheral nerve dysfunction or other indirect causes (Van Raamsdonk et al. 2007; Van der Burg et al. 2009) which require further investigation.

11 Mechanisms of Neuropathology

The exact mechanisms of neuronal cell death in HD are currently unclear. What has been observed in recent neuroimaging studies is that the neuropathological changes are occurring up to 10 years before clinical diagnosis and striatal atrophy becomes more severe closer to clinical onset and clinical onset can be predicted within about 2 years (Aylward et al. 2004; Bohanna et al. 2008). Even though only one gene is mutated in HD, the genetics of HD are extremely complex. The expanded CAG repeat of the *HTT* gene is expected to interact with large numbers of other genes as evidenced by the results of gene microarray studies showing large numbers of affected genes in studies on both postmortem HD tissue (Hodges et al. 2006) and mouse models of HD (Luthi-Carter et al. 2000). These interactions lead to a complex set of parameters that may involve transcriptional dysregulation, excitotoxicity, oxidative stress, changes in neurotransmitters, disruption of cortical BDNF production, and breakdown of cellular and vesicular transport mechanisms in neurons of the striatum, cerebral cortex, and other regions throughout the brain (Cha 2000; Cattaneo et al. 2001; Morton et al. 2001; Zuccato and Cattaneo 2007; Rosas et al. 2008; Thu et al. 2010). In the striatum, it is the medium spiny neurons which are the most vulnerable, particularly the subset of enkephalin-containing striatopallidal neurons which are found throughout the striatum; however, the loss of these neurons can be quite variable in relation to the striosome-matrix compartments. Recent transgenic animal studies have implicated dysfunction of the cortex as one of the major indicators of phenotype; this may occur through cortical synaptic dysfunction even before cell death (Cepeda et al. 2007; Cummings et al. 2009) and that dysfunction of the corticostriatal neurons could lead to anterograde neurodegeneration of striatal neurons. Also, abnormal glutamate receptor functions in the cerebral cortex have been implicated in behavioral and motor impairments in transgenic mice with physiological and morphological cortical changes predicting the onset and severity of behavioral deficits (Sapp et al. 1997; Laforet et al. 2001;

Andre et al. 2006). Furthermore, studies in the conditional mouse model where cortical and/or striatal cells selectively express mHtt showed that dysfunction of the cortical neurons was essential to the development of significant behavioral and motor deficits (Gu et al. 2007). Other transgenic mouse studies have implicated dysfunction of both the cortical projection and interneurons of the cerebral cortex in the development of HD pathology (Gu et al. 2005; Spampanato et al. 2008). All of these animal studies provide accumulating mechanistic evidence that the cortex plays a major role in the initiation and development of the HD phenotype and that dysfunction in the corticostriate neurons plays a major role in HD forebrain pathology. The dysfunction of the growth factor BDNF in the glutamatergic cortico-striatal pyramidal neurons has also been implicated in either causing the death of these pyramidal neurons and/or causing a dysfunction of their firing resulting in excess glutamate release causing the death of striatal neurons (Cepeda et al. 2007; Strand et al. 2007; Zuccato and Cattaneo 2007). It has long been known that the cerebral cortex is not a homogeneous structure as evidenced by the different morphological compositions of the Brodmann areas. Furthermore, genetic studies show that neurons in the different regions of the cerebral cortex have a variable genetic expression profile which defines their particular subtype (Molyneaux et al. 2007). This suggests that neurons throughout the brain but particularly in specific cell types throughout the different regions of the cerebral cortex and basal ganglia may interact differently with the mutant *HTT* gene and cause degeneration in variable populations of pyramidal neurons, cortical interneurons, and striatal neurons. This could be an underlying factor in the major susceptibility of the human forebrain to the HD process as well as regional and cellular variability observed within the various regions of the human forebrain.

The neuropathology of HD is constantly being re-evaluated. The most recent studies have shown that the neurodegeneration throughout the brain is highly heterogeneous. Although the most severe pathology occurs in the basal ganglia and cerebral cortex, in the striatum, there is a continuum of degeneration related to the striosome and matrix compartments, in the cerebral cortex, there is a highly variable distribution of degeneration of neurons and related gliosis, and regions of the brain outside of the basal ganglia and cortex such as the thalamus, hypothalamus, cerebellum, and brainstem still await detailed investigation. Additionally, the various inputs to the striatum which have been described recently such as feedback loops from the GPe (Bevan et al. 1998), the thalamic intralaminar nuclei (Smith et al. 2004), and the hyperdirect pathway from the cortex to the STN (Nambu et al. 2002) may all influence basal ganglia pathways in complex ways. The heterogeneous nature of the symptomatology of HD is clearly associated with the heterogeneous nature of the neurodegeneration and major pathways that occur throughout the different regions of the brain in different individuals, and the great challenge is to relate this pattern of heterogeneity to the mutant genotype and its variable effects on gene expression profiles across the entire human genome.

References

Albin RL, Reiner A, Anderson KD, Dure LS, Handelin B, Balfour R, Whetsell WO Jr, Penney JB, Young AB (1992) Preferential loss of striato-external pallidal projection neurons in presymptomatic Huntington's disease. Ann Neurol 31(4):425–430

Albin RL, Reiner A, Anderson KD, Penney JB, Young AB (1990) Striatal and nigral neuron subpopulations in rigid Huntington's disease: implications for the functional anatomy of chorea and rigidity-akinesia. Ann Neurol 27(4):357–365

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12(10):366–375

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13(7):266–271

Allen KL, Waldvogel HJ, Glass M, Faull RLM (2009) Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. J Chem Neuroanat 37(4):266–281

Andre VM, Cepeda C, Venegas A, Gomez Y, Levine MS (2006) Altered cortical glutamate receptor function in the R6/2 model of Huntington's disease. J Neurophysiol 95(4):2108–2119

Andreassen OA, Dedeoglu A, Stanojevic V, Hughes DB, Browne SE, Leech CA, Ferrante RJ, Habener JF, Beal MF, Thomas MK (2002) Huntington's disease of the endocrine pancreas: insulin deficiency and diabetes mellitus due to impaired insulin gene expression. Neurobiol Dis 11(3):410–424

Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B, Kalchman MA et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4(4):398–403

Arenas J, Campos Y, Ribacoba R, Martin MA, Rubio JC, Ablanedo P, Cabello A (1998) Complex I defect in muscle from patients with Huntington's disease. Ann Neurol 43(3):397–400

Aronin N, Chase K, Young C, Sapp E, Schwarz C, Matta N, Kornreich R, Landwehrmeyer B, Bird E, Beal MF et al (1995) CAG expansion affects the expression of mutant Huntingtin in the Huntington's disease brain. Neuron 15(5):1193–1201

Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431(7010):805–810

Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R (2007) Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. Hum Mol Genet 16(21):2600–2615

Augood SJ, Faull RLM, Love DR, Emson PC (1996) Reduction in enkephalin and substance P messenger RNA in the striatum of early grade Huntington's disease: a detailed cellular in situ hybridization study. Neuroscience 72(4):1023–1036

Aylward EH, Anderson NB, Bylsma FW, Wagster MV, Barta PE, Sherr M, Feeney J, Davis A, Rosenblatt A, Pearlson GD, Ross CA (1998) Frontal lobe volume in patients with Huntington's disease. Neurology 50(1):252–258

Aylward EH, Li Q, Stine OC, Ranen N, Sherr M, Barta PE, Bylsma FW, Pearlson GD, Ross CA (1997) Longitudinal change in basal ganglia volume in patients with Huntington's disease. Neurology 48(2):394–399

Aylward EH, Sparks BF, Field KM, Yallapragada V, Shpritz BD, Rosenblatt A, Brandt J, Gourley LM, Liang K, Zhou H, Margolis RL, Ross CA (2004) Onset and rate of striatal atrophy in preclinical Huntington disease. Neurology 63(1):66–72

Aziz A, Fronczek R, Maat-Schieman M, Unmehopa U, Roelandse F, Overeem S, van Duinen S, Lammers GJ, Swaab D, Roos R (2008) Hypocretin and melanin-concentrating hormone in patients with Huntington disease. Brain Pathol 18(4):474–483

Beal MF (1994) Huntington's disease, energy, and excitotoxicity. Neurobiol Aging 15(2):275–276

Becher MW, Kotzuk JA, Sharp AH, Davies SW, Bates GP, Price DL, Ross CA (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol Dis 4(6):387–397

Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18(22):9438–9452

Bhatia KP, Marsden CD (1994) The behavioural and motor consequences of focal lesions of the basal ganglia in man. Brain 117(Pt 4):859–876

Bird ED, Iversen LL (1974) Huntington's chorea. Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain 97(3):457–472

Birnbaum G (1941) Chronisch-progressive Chorea mit Kleinhirnatrophie. Archiv für Psychiatrie und Nervenkrankheiten 114:160–182

Bohanna I, Georgiou-Karistianis N, Hannan AJ, Egan GF (2008) Magnetic resonance imaging as an approach towards identifying neuropathological biomarkers for Huntington's disease. Brain Res Rev 58(1):209–225

Brandt J, Butters N (1986) The neuropsychology of Huntington's disease. Trends Neurosci 9:118–120

Bryun GW (1968) Huntington's chorea; historical, clinical and laboratory synopsis. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol 6. North Holland, Amsterdam, pp 379–396

Byers RK, Gilles FH, Fung C (1973) Huntington's disease in children. Neuropathologic study of four cases. Neurology 23(6):561–569

Campbell AM, Corner B, Norman RM, Urich H (1961) The rigid form of Huntington's disease. J Neurol Neurosurg Psychiatry 24:71–77

Carpenter MB, Nakano K, Kim R (1976) Nigrothalamic projections in the monkey demonstrated by autoradiographic technics. J Comp Neurol 165(4):401–415

Cattaneo E, Rigamonti D, Goffredo D, Zuccato C, Squitieri F, Sipione S (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. Trends Neurosci 24 (3):182–188

Cepeda C, Wu N, Andre VM, Cummings DM, Levine MS (2007) The corticostriatal pathway in Huntington's disease. Prog Neurobiol 81(5–6):253–271

Cha JH (2000) Transcriptional dysregulation in Huntington's disease. Trends Neurosci 23 (9):387–392

Cicchetti F, Parent A (1996) Striatal interneurons in Huntington's disease: selective increase in the density of calretinin-immunoreactive medium-sized neurons. Mov Disord 11(6):619–626

Cicchetti F, Prensa L, Wu Y, Parent A (2000) Chemical anatomy of striatal interneurons in normal individuals and in patients with Huntington's disease. Brain Res Brain Res Rev 34(1–2):80–101

Claes S, Van Zand K, Legius E, Dom R, Malfroid M, Baro F, Godderis J, Cassiman JJ (1995) Correlations between triplet repeat expansion and clinical features in Huntington's disease. Arch Neurol 52(8):749–753

Cudkowicz M, Kowall NW (1990a) Degeneration of pyramidal projection neurons in Huntington's disease cortex. Ann Neurol 27(2):200–204

Cudkowicz M, Kowall NW (1990b) Parvalbumin immunoreactive neurons are resistant to degeneration in Huntington's disease cerebral cortex. J Neuropathol Exp Neurol 49:345

Cummings DM, Andre VM, Uzgil BO, Gee SM, Fisher YE, Cepeda C, Levine MS (2009) Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. J Neurosci 29(33):10371–10386

Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, Holtas S, van Roon-Mom WM, Bjork-Eriksson T, Nordborg C, Frisen J, Dragunow M, Faull RLM, Eriksson PS (2007) Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315(5816):1243–1249

Curtis MA, Low VF, Faull RLM (2012) Neurogenesis and progenitor cells in the adult human brain: a comparison between hippocampal and subventricular progenitor proliferation. Dev Neurobiol 72(7):990–1005

Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, Dragunow M, Connor B, Faull RLM (2003) Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc Natl Acad Sci U S A 100(15):9023–9027

Curtis MA, Penney EB, Pearson J, Dragunow M, Connor B, Faull RLM (2005) The distribution of progenitor cells in the subependymal layer of the lateral ventricle in the normal and Huntington's disease human brain. Neuroscience 132(3):777–788

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90(3):537–548

Davies SW, Turmaine M, Cozens BA, Raza AS, Mahal A, Mangiarini L, Bates GP (1999) From neuronal inclusions to neurodegeneration: neuropathological investigation of a transgenic mouse model of Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354(1386):981–989

Dawbarn D, De Quidt ME, Emson PC (1985) Survival of basal ganglia neuropeptide Y-somatostatin neurones in Huntington's disease. Brain Res 340(2):251–260

de la Monte SM, Vonsattel JP, Richardson EP Jr (1988) Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. J Neuropathol Exp Neurol 47(5):516–525

DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13(7):281–285

Deng YP, Albin RL, Penney JB, Young AB, Anderson KD, Reiner A (2004) Differential loss of striatal projection systems in Huntington's disease: a quantitative immunohistochemical study. J Chem Neuroanat 27(3):143–164

Deng YP, Wong T, Bricker-Anthony C, Deng B, Reiner A (2013) Loss of corticostriatal and thalamostriatal synaptic terminals precedes striatal projection neuron pathology in heterozygous Q140 Huntington's disease mice. Neurobiol Dis 60:89–107

Di Maio L, Squitieri F, Napolitano G, Campanella G, Trofatter JA, Conneally PM (1993) Onset symptoms in 510 patients with Huntington's disease. J Med Genet 30(4):289–292

DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, Martin E, Vonsattel JP, Carraway R, Reeves SA et al (1995) Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 14(5):1075–1081

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277(5334):1990–1993

Doig NM, Moss J, Bolam JP (2010) Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. J Neurosci 30(44):14610–14618

Douaud G, Gaura V, Ribeiro MJ, Lethimonnier F, Maroy R, Verny C, Krystkowiak P, Damier P, Bachoud-Levi AC, Hantraye P, Remy P (2006) Distribution of grey matter atrophy in Huntington's disease patients: a combined ROI-based and voxel-based morphometric study. Neuroimage 32(4):1562–1575

Dunlap CB (1927) Pathologic changes in Huntington's chorea with special reference to the corpus striatum. Arch Neurol Psychiat 18:867–943

Dure LS, Young AB, Penney JB (1991) Excitatory amino acid binding sites in the caudate nucleus and frontal cortex of Huntington's disease. Ann Neurol 30 (6):785–793

Dure LS, Young AB, Penney JB, Jr. (1992) Compartmentalization of excitatory amino acid receptors in human striatum. Proc Natl Acad Sci U S A 89 (16):7688–7692

Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel JP, Gusella JF, Joyner AL et al (1995) Inactivation of the mouse Huntington's disease gene homolog Hdh. Science 269(5222):407–410

Emson PC, Arregui A, Clement-Jones V, Sandberg BE, Rossor M (1980) Regional distribution of methionine-enkephalin and substance P-like immunoreactivity in normal human brain and in Huntington's disease. Brain Res 199(1):147–160

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4(11):1313–1317

Faull RLM, Mehler WR (1978) The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. Neuroscience 3(11):989–1002

Faull RLM, Villiger JW (1986) Heterogeneous distribution of benzodiazepine receptors in the human striatum: a quantitative autoradiographic study comparing the pattern of receptor labelling with the distribution of acetylcholinesterase staining. Brain Res 381(1):153–158

Faull RLM, Waldvogel HJ, Nicholson LF, Synek BJ (1993) The distribution of GABAA-benzodiazepine receptors in the basal ganglia in Huntington's disease and in the quinolinic acid-lesioned rat. Prog Brain Res 99:105–123

Ferrante RJ, Gutekunst CA, Persichetti F, McNeil SM, Kowall NW, Gusella JF, MacDonald ME, Beal MF, Hersch SM (1997) Heterogeneous topographic and cellular distribution of huntingtin expression in the normal human neostriatum. J Neurosci 17(9):3052–3063

Ferrante RJ, Kowall NW (1987) Tyrosine hydroxylase-like immunoreactivity is distributed in the matrix compartment of normal human and Huntington's disease striatum. Brain Res 416 (1):141–146

Ferrante RJ, Kowall NW, Beal MF, Martin JB, Bird ED, Richardson EP Jr (1987) Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease. J Neuropathol Exp Neurol 46(1):12–27

Ferrante RJ, Kowall NW, Richardson EP Jr (1989) Neuronal and neuropil loss in the substantia nigra in Huntington's disease. J Neuropath Exp Neurol 48:380

Ferrante RJ, Kowall NW, Richardson EP Jr (1991) Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry. J Neurosci 11(12):3877–3887

Ferrer I, Kulisevsky J, Gonzalez G, Escartin A, Chivite A, Casas R (1994) Parvalbumin-immunoreactive neurons in the cerebral cortex and striatum in Huntington's disease. Neurodegeneration 3:169–173

Folstein SE (1989) Huntington's disease: a disorder of families. Johns Hopkins University Press, Baltimore

Forno LS, Jose C (1973) Huntington's chorea: A pathological study. In: Barbeau A, Chase TN, Paulson GW (eds) Adv Neurol, vol 1., vol 1Raven, New York, pp 453–470

Friedman JH, Trieschmann ME, Myers RH, Fernandez HH (2005) Monozygotic twins discordant for Huntington disease after 7 years. Arch Neurol 62(6):995–997

Fujiyama F, Sohn J, Nakano T, Furuta T, Nakamura KC, Matsuda W, Kaneko T (2011) Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector. Eur J Neurosci 33(4):668–677

Fusco FR, Chen Q, Lamoreaux WJ, Figueredo-Cardenas G, Jiao Y, Coffman JA, Surmeier DJ, Honig MG, Carlock LR, Reiner A (1999) Cellular localization of huntingtin in striatal and cortical neurons in rats: lack of correlation with neuronal vulnerability in Huntington's disease. J Neurosci 19(4):1189–1202

Georgiou N, Bradshaw JL, Chiu E, Tudor A, O'Gorman L, Phillips JG (1999) Differential clinical and motor control function in a pair of monozygotic twins with Huntington's disease. Mov Disord 14(2):320–325

Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311(5985):461–464

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250(4986):1429–1432

Glass M, Dragunow M, Faull RLM (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97(3):505–519

Glass M, Faull RLM, Dragunow M (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. Neuroscience 56(3):523–527

Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, Bromm M, Kazemi-Esfarjani P, Thornberry NA, Vaillancourt JP, Hayden MR (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. Nat Genet 13(4):442–449

Gomez-Esteban JC, Lezcano E, Zarranz JJ, Velasco F, Garamendi I, Perez T, Tijero B (2007) Monozygotic twins suffering from Huntington's disease show different cognitive and behavioural symptoms. Eur Neurol 57(1):26–30

Gomez-Tortosa E, Irizarry MC, Gomez-Isla T, Hyman BT (2000) Clinical and neuropathological correlates of dementia with Lewy bodies. Ann N Y Acad Sci 920:9–15

Gomez-Tortosa E, MacDonald ME, Friend JC, Taylor SA, Weiler LJ, Cupples LA, Srinidhi J, Gusella JF, Bird ED, Vonsattel JP, Myers RH (2001) Quantitative neuropathological changes in presymptomatic Huntington's disease. Ann Neurol 49(1):29–34

Gong B, Lim MC, Wanderer J, Wyttenbach A, Morton AJ (2008) Time-lapse analysis of aggregate formation in an inducible PC12 cell model of Huntington's disease reveals time-dependent aggregate formation that transiently delays cell death. Brain Res Bull 75(1):146–157

Goto S, Hirano A, Rojas-Corona RR (1989) An immunohistochemical investigation of the human neostriatum in Huntington's disease. Ann Neurol 25(3):298–304

Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13(7):244–254

Graybiel AM (1995) The basal ganglia. Trends Neurosci 18(2):60–62

Graybiel AM, Ragsdale CW Jr (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci U S A 75(11):5723–5726

Graybiel AM, Ragsdale CW Jr, Edley SM (1979) Compartments in the striatum of the cat observed by retrograde cell labeling. Exp Brain Res 34(1):189–195

Greenamyre JT, Penney JB, Young AB, D'Amato CJ, Hicks SP, Shoulson I (1985) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. Science 227(4693):1496–1499

Gu X, Andre VM, Cepeda C, Li SH, Li XJ, Levine MS, Yang XW (2007) Pathological cell-cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. Mol Neurodegener 2:8

Gu X, Li C, Wei W, Lo V, Gong S, Li SH, Iwasato T, Itohara S, Li XJ, Mody I, Heintz N, Yang XW (2005) Pathological cell-cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron 46(3):433–444

Guo Z, Rudow G, Pletnikova O, Codispoti KE, Orr BA, Crain BJ, Duan W, Margolis RL, Rosenblatt A, Ross CA, Troncoso JC (2012) Striatal neuronal loss correlates with clinical motor impairment in Huntington's disease. Mov Disord 27(11):1379–1386

Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci 19(7):2522–2534

Hadzi TC, Hendricks AE, Latourelle JC, Lunetta KL, Cupples LA, Gillis T, Mysore JS, Gusella JF, Macdonald ME, Myers RH, Vonsattel JP (2012) Assessment of cortical and striatal involvement in 523 Huntington disease brains. Neurology

Hallervorden J (1957) Huntingtonsche Chorea (Chorea chronica progressiva hereditaria). Handbuch der speziellen pathologischen Anatomie und Histologie (XIII/1 Bandteil A). Springer Verlag, Berlin. Göttingen. Heidelberg, pp 793–822

Halliday GM, McRitchie DA, Macdonald V, Double KL, Trent RJ, McCusker E (1998) Regional specificity of brain atrophy in Huntington's disease. Experimental Neurology 154(2):663–672

Harper PS, Morris MR, Quarrell OWJ, Shaw DJ, Tyler A, Youngman S (1991) The clinical neurology of Huntington's disease. Huntington's Disease Major Problems in Neurology, 22. W.B. Saunders Company LTD, London Philadelphia Toronto Sydney Tokyo, pp 37–80

Harrington KM, Kowall NW (1991) Parvalbumin immunoreactive neurons resist degeneration in Huntington's disease striatum. J Neuropath Exp Neurol 50:309

Hedreen JC, Folstein SE (1995) Early loss of neostriatal striosome neurons in Huntington's disease. J Neuropathol Exp Neurol 54(1):105–120

Hedreen JC, Peyser CE, Folstein SE, Ross CA (1991) Neuronal loss in layers V and VI of cerebral cortex in Huntington's disease. Neuroscience Letters 133(2):257–261

Heinsen H, Rub U, Bauer M, Ulmar G, Bethke B, Schuler M, Bocker F, Eisenmenger W, Gotz M, Korr H, Schmitz C (1999) Nerve cell loss in the thalamic mediodorsal nucleus in Huntington's disease. Acta Neuropathol 97(6):613–622

Heinsen H, Rub U, Gangnus D, Jungkunz G, Bauer M, Ulmar G, Bethke B, Schuler M, Bocker F, Eisenmenger W, Gotz M, Strik M (1996) Nerve cell loss in the thalamic centromedian-parafascicular complex in patients with Huntington's disease. Acta Neuropathol 91(2):161–168

Heinsen H, Strik M, Bauer M, Luther K, Ulmar G, Gangnus D, Jungkunz G, Eisenmenger W, Gotz M (1994) Cortical and striatal neurone number in Huntington's disease. Acta Neuropathol 88(4):320–333

Herndon ES, Hladik CL, Shang P, Burns DK, Raisanen J, White CL 3rd (2009) Neuroanatomic profile of polyglutamine immunoreactivity in Huntington disease brains. J Neuropathol Exp Neurol 68(3):250–261

Hilditch-Maguire P, Trettel F, Passani LA, Auerbach A, Persichetti F, MacDonald ME (2000) Huntingtin: an iron-regulated protein essential for normal nuclear and perinuclear organelles. Hum Mol Genet 9(19):2789–2797

Hobbs NZ, Pedrick AV, Say MJ, Frost C, Santos RD, Coleman A, Sturrock A, Craufurd D, Stout JC, Leavitt BR, Barnes J, Tabrizi SJ, Scahill RI (2011) The structural involvement of the cingulate cortex in premanifest and early Huntington's disease. Mov Disord 26(9):1684–1690

Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RLM, Olson JM, Jones L, Luthi-Carter R (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15(6):965–977

Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, Smith DJ, Bissada N, McCutcheon K, Nasir J, Jamot L, Li XJ, Stevens ME, Rosemond E, Roder JC, Phillips AG, Rubin EM, Hersch SM, Hayden MR (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 23(1):181–192

Holt DJ, Graybiel AM, Saper CB (1997) Neurochemical architecture of the human striatum. J Comp Neurol 384(1):1–25

Holt DJ, Hersh LB, Saper CB (1996) Cholinergic innervation in the human striatum: a three-compartment model. Neuroscience 74(1):67–87

Huang K, Yanai A, Kang R, Arstikaitis P, Singaraja RR, Metzler M, Mullard A, Haigh B, Gauthier-Campbell C, Gutekunst CA, Hayden MR, El-Husseini A (2004) Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. Neuron 44(6):977–986

Hult S, Schultz K, Soylu R, Petersen A (2010) Hypothalamic and neuroendocrine changes in Huntington's disease. Curr Drug Targets 11(10):1237–1249

Hunt MJ, Morton AJ (2005) Atypical diabetes associated with inclusion formation in the R6/2 mouse model of Huntington's disease is not improved by treatment with hypoglycaemic agents. Exp Brain Res 166(2):220–229

Huntington Study Group (1996) Unified Huntington's Disease Rating Scale: reliability and consistency. Mov Disord 11(2):136–142

Jernigan TL, Salmon DP, Butters N, Hesselink JR (1991) Cerebral structure on MRI, Part II: Specific changes in Alzheimer's and Huntington's diseases. Biol Psychiatry 29(1):68–81

Jervis GA (1963) Huntington's Chorea in childhood. Arch Neurol 9:244–257

Jeste DV, Barban L, Parisi J (1984) Reduced Purkinje cell density in Huntington's disease. Exp Neurol 85(1):78–86

Joyce JN, Lexow N, Bird E, Winokur A (1988) Organization of dopamine D1 and D2 receptors in human striatum: receptor autoradiographic studies in Huntington's disease and schizophrenia. Synapse 2(5):546–557

Kaltenbach LS, Romero E, Becklin RR, Chettier R, Bell R, Phansalkar A, Strand A, Torcassi C, Savage J, Hurlburt A, Cha GH, Ukani L, Chepanoske CL, Zhen Y, Sahasrabudhe S, Olson J, Kurschner C, Ellerby LM, Peltier JM, Botas J, Hughes RE (2007) Huntingtin interacting proteins are genetic modifiers of neurodegeneration. PLoS Genet 3(5):e82

Kam M, Curtis MA, McGlashan SR, Connor B, Nannmark U, Faull RLM (2009) The cellular composition and morphological organization of the rostral migratory stream in the adult human brain. J Chem Neuroanat 37(3):196–205

Kassubek J, Gaus W, Landwehrmeyer GB (2004) Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. Neurology 62(3):523–524

Kassubek J, Juengling FD, Ecker D, Landwehrmeyer GB (2005) Thalamic atrophy in Huntington's disease co-varies with cognitive performance: a morphometric MRI analysis. Cereb Cortex 15(6):846–853

Kayahara T, Nakano K (1996) Pallido-thalamo-motor cortical connections: an electron microscopic study in the macaque monkey. Brain Res 706(2):337–342

Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, Sapp E, Wang Y, Qin ZH, Chen JD, Nevins JR, Aronin N, DiFiglia M (2002) Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J Biol Chem 277(9):7466–7476

Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, De La Calle A (1998) Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. J Comp Neurol 402(3):353–371

Kiesselbach G (1914) Anatomischer Befund eines Falles von Huntingtonscher Chorea. MonatsschrPsychiatNeurol 35:525–543

Kitamura A, Kubota H, Pack CG, Matsumoto G, Hirayama S, Takahashi Y, Kimura H, Kinjo M, Morimoto RI, Nagata K (2006) Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. Nat Cell Biol 8(10):1163–1170

Kowall NW, Quigley BJ Jr, Krause JE, Lu F, Kosofsky BE, Ferrante RJ (1993) Substance P and substance P receptor histochemistry in human neurodegenerative diseases. Regul Pept 46 (1–2):174–185

Kremer HP (1992) The hypothalamic lateral tuberal nucleus: normal anatomy and changes in neurological diseases. Prog Brain Res 93:249–261

Kremer HP, Roos RA, Dingjan G, Marani E, Bots GT (1990) Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease. J Neuropathol Exp Neurol 49(4):371–382

Kremer HP, Roos RA, Dingjan GM, Bots GT, Bruyn GW, Hofman MA (1991) The hypothalamic lateral tuberal nucleus and the characteristics of neuronal loss in Huntington's disease. Neurosci Lett 132(1):101–104

Kuemmerle S, Gutekunst CA, Klein AM, Li XJ, Li SH, Beal MF, Hersch SM, Ferrante RJ (1999) Huntington aggregates may not predict neuronal death in Huntington's disease. Ann Neurol 46 (6):842–849

Kuppenbender KD, Standaert DG, Feuerstein TJ, Penney JB Jr, Young AB, Landwehrmeyer GB (2000) Expression of NMDA receptor subunit mRNAs in neurochemically identified projection and interneurons in the human striatum. J Comp Neurol 419(4):407–421

Laforet GA, Sapp E, Chase K, McIntyre C, Boyce FM, Campbell M, Cadigan BA, Warzecki L, Tagle DA, Reddy PH, Cepeda C, Calvert CR, Jokel ES, Klapstein GJ, Ariano MA, Levine MS, DiFiglia M, Aronin N (2001) Changes in cortical and striatal neurons predict behavioral and electrophysiological abnormalities in a transgenic murine model of Huntington's disease. J Neurosci 21(23):9112–9123

Lalic NM, Maric J, Svetel M, Jotic A, Stefanova E, Lalic K, Dragasevic N, Milicic T, Lukic L, Kostic VS (2008) Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. Arch Neurol 65(4):476–480

Landles C, Bates GP (2004) Huntingtin and the molecular pathogenesis of Huntington's disease. Fourth in molecular medicine review series. EMBO Rep 5(10):958–963

Landles C, Sathasivam K, Weiss A, Woodman B, Moffitt H, Finkbeiner S, Sun B, Gafni J, Ellerby LM, Trottier Y, Richards WG, Osmand A, Paganetti P, Bates GP (2010) Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. J Biol Chem 285(12):8808–8823

Lange H, Thorner G, Hopf A, Schroder KF (1976) Morphometric studies of the neuropathological changes in choreatic diseases. J Neurol Sci 28(4):401–425

Lanska DJ, Lanska MJ, Lavine L, Schoenberg BS (1988) Conditions associated with Huntington's disease at death. A case-control study. Arch Neurol 45(8):878–880

Lewy FH (1923) Die Histopathologie der choreatischen Erkrankungen. Zeitschrift für die gesamte Neurologie und Psychiatrie (Berlin) 85:622–658

Li JY, Plomann M, Brundin P (2003) Huntington's disease: a synaptopathy? Trends Mol Med 9 (10):414–420

Li SH, Li XJ (2004) Huntingtin-protein interactions and the pathogenesis of Huntington's disease. Trends Genet 20(3):146–154

Lievens JC, Iche M, Laval M, Faivre-Sarrailh C, Birman S (2008) AKT-sensitive or insensitive pathways of toxicity in glial cells and neurons in Drosophila models of Huntington's disease. Hum Mol Genet 17(6):882–894

Low VF, Dragunow M, Tippett LJ, Faull RLM, Curtis MA (2011) No change in progenitor cell proliferation in the hippocampus in Huntington's disease. Neuroscience 199:577–588

Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, Mandel JL, Trottier Y (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. Mol Cell 10(2):259–269

Luthi-Carter R, Strand A, Peters NL, Solano SM, Hollingsworth ZR, Menon AS, Frey AS, Spektor BS, Penney EB, Schilling G, Ross CA, Borchelt DR, Tapscott SJ, Young AB, Cha JH, Olson JM (2000) Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. Hum Mol Genet 9(9):1259–1271

Maat-Schieman ML, Dorsman JC, Smoor MA, Siesling S, Van Duinen SG, Verschuuren JJ, den Dunnen JT, Van Ommen GJ, Roos RA (1999) Distribution of inclusions in neuronal nuclei and dystrophic neurites in Huntington disease brain. J Neuropathol Exp Neurol 58(2):129–137

Macdonald V, Halliday GM (2002) Pyramidal cell loss in motor cortices in Huntington's disease. Neurobiology of Disease 10(3):378–386

Macdonald V, Halliday GM, Trent RJ, McCusker EA (1997) Significant loss of pyramidal neurons in the angular gyrus of patients with Huntington's disease. Neuropathol Appl Neurobiol 23 (6):492–495

Machida Y, Okada T, Kurosawa M, Oyama F, Ozawa K, Nukina N (2006) rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. Biochem Biophys Res Commun 343(1):190–197

MacMillan JC, Snell RG, Tyler A, Houlihan GD, Fenton I, Cheadle JP, Lazarou LP, Shaw DJ, Harper PS (1993) Molecular analysis and clinical correlations of the Huntington's disease mutation. Lancet 342(8877):954–958

Manley MS, Young SJ, Groves PM (1994) Compartmental organization of the peptide network in the human caudate nucleus. J Chem Neuroanat 7(3):191–201

Markham CH, Knox JW (1965) Observations on Huntington's Chorea in Childhood. J Pediatr 67:46–57

Marshall PE, Landis DM, Zalneraitis EL (1983) Immunocytochemical studies of substance P and leucine-enkephalin in Huntington's disease. Brain Res 289(1–2):11–26

Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, McCutcheon K, Singaraja R, Kazemi-Esfarjani P, Devon R, Kim SU, Bredesen DE, Tufaro F, Hayden MR (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nat Genet 18(2):150–154

McCaughey WTE (1961) The pathologic spectrum of Huntington's chorea. J Nerv Ment Dis 133:91–103

McGeorge AJ, Faull RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29(3):503–537

Mehler WR (1971) Idea of a new anatomy of the thalamus. J Psychiatr Res 8(3):203–217
Mena-Segovia J, Bolam JP, Magill PJ (2004) Pedunculopontine nucleus and basal ganglia: distant relatives or part of the same family? Trends Neurosci 27(10):585–588
Molyneaux BJ, Arlotta P, Menezes JR, Macklis JD (2007) Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci 8(6):427–437
Montoya A, Price BH, Menear M, Lepage M (2006) Brain imaging and cognitive dysfunctions in Huntington's disease. J Psychiatry Neurosci 31(1):21–29
Morton AJ, Faull RLM, Edwardson JM (2001) Abnormalities in the synaptic vesicle fusion machinery in Huntington's disease. Brain Res Bull 56(2):111–117
Morton AJ, Nicholson LF, Faull RLM (1993) Compartmental loss of NADPH diaphorase in the neuropil of the human striatum in Huntington's disease. Neuroscience 53(1):159–168
Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES (2005) Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J Neurosci 25(1):157–163
Myers RH, Sax DS, Koroshetz WJ, Mastromauro C, Cupples LA, Kiely DK, Pettengill FK, Bird ED (1991a) Factors associated with slow progression in Huntington's disease. Arch Neurol 48 (8):800–804
Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, Richardson EP Jr, Bird ED (1991b) Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. J Neuropathol Exp Neurol 50(6):729–742
Nambu A, Tokuno H, Takada M (2002) Functional significance of the cortico-subthalamo-pallidal 'hyperdirect' pathway. Neurosci Res 43(2):111–117
Nauta WJ, Domesick VB (1984) Afferent and efferent relationships of the basal ganglia. Ciba Found Symp 107:3–29
Neustaedter M (1933) Concerning the striatal localization in chronic progressive chorea. With a report of three cases, two of the Huntington type in siblings and one senile arteriosclerotic, with necropsies. Journal of Nervous and Mental Disease 78:470–491
Nopoulos P, Aylward EH, Ross CA, Johnson HJ, Magnotta VA, Juhl AR, Pierson RK, Mills J, Langbehn DR, Paulsen JS (2010) Cerebral cortex structure in prodromal Huntington disease. Neurobiol Dis 40(3):544–554
Nopoulos P, Magnotta VA, Mikos A, Paulson H, Andreasen NC, Paulsen JS (2007) Morphology of the cerebral cortex in preclinical Huntington's disease. Am J Psychiatry 164(9):1428–1434
Ouimet CC, Langley-Gullion KC, Greengard P (1998) Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. Brain Res 808(1):8–12
Oyanagi K, Ikuta F (1987) A morphometric reevaluation of Huntington's chorea with special reference to the large neurons in the neostriatum. Clin Neuropathol 6(2):71–79
Oyanagi K, Takeda S, Takahashi H, Ohama E, Ikuta F (1989) A quantitative investigation of the substantia nigra in Huntington's disease. Ann Neurol 26(1):13–19
Parent A, Cote PY, Lavoie B (1995) Chemical anatomy of primate basal ganglia. Prog Neurobiol 46(2–3):131–197
Parent A, Fortin M, Cote PY, Cicchetti F (1996) Calcium-binding proteins in primate basal ganglia. Neurosci Res 25(4):309–334
Parent A, Hazrati LN (1993) Anatomical aspects of information processing in primate basal ganglia. Trends Neurosci 16(3):111–116
Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 20(1):91–127
Paulsen JS (2009) Functional imaging in Huntington's disease. Exp Neurol 216(2):272–277
Pavese N, Gerhard A, Tai YF, Ho AK, Turkheimer F, Barker RA, Brooks DJ, Piccini P (2006) Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology 66(11):1638–1643
Penney JB Jr, Young AB (1982) Quantitative autoradiography of neurotransmitter receptors in Huntington disease. Neurology 32(12):1391–1395
Perutz MF (1996) Glutamine repeats and inherited neurodegenerative diseases: molecular aspects. Curr Opin Struct Biol 6(6):848–858

Perutz MF, Johnson T, Suzuki M, Finch JT (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. Proc Natl Acad Sci USA 91(12):5355–5358

Petersen A, Bjorkqvist M (2006) Hypothalamic-endocrine aspects in Huntington's disease. Eur J Neurosci 24(4):961–967

Petersen A, Gil J, Maat-Schieman ML, Bjorkqvist M, Tanila H, Araujo IM, Smith R, Popovic N, Wierup N, Norlen P, Li JY, Roos RA, Sundler F, Mulder H, Brundin P (2005) Orexin loss in Huntington's disease. Hum Mol Genet 14(1):39–47

Pillai JA, Hansen LA, Masliah E, Goldstein JL, Edland SD, Corey-Bloom J (2012) Clinical severity of Huntington's disease does not always correlate with neuropathologic stage. Mov Disord 27(9):1099–1103

Podolsky S, Leopold NA, Sax DS (1972) Increased frequency of diabetes mellitus in patients with Huntington's chorea. Lancet 1(7765):1356–1358

Politis M, Pavese N, Tai YF, Tabrizi SJ, Barker RA, Piccini P (2008) Hypothalamic involvement in Huntington's disease: an in vivo PET study. Brain 131(Pt 11):2860–2869

Prensa L, Gimenez-Amaya JM, Parent A (1999) Chemical heterogeneity of the striosomal compartment in the human striatum. J Comp Neurol 413(4):603–618

Rajkowska G, Selemon LD, Goldman-Rakic PS (1998) Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. Arch Gen Psychiatry 55(3):215–224

Ratovitski T, Gucek M, Jiang H, Chighladze E, Waldron E, D'Ambola J, Hou Z, Liang Y, Poirier MA, Hirschhorn RR, Graham R, Hayden MR, Cole RN, Ross CA (2009) Mutant huntingtin N-terminal fragments of specific size mediate aggregation and toxicity in neuronal cells. J Biol Chem 284(16):10855–10867

Reading SA, Yassa MA, Bakker A, Dziorny AC, Gourley LM, Yallapragada V, Rosenblatt A, Margolis RL, Aylward EH, Brandt J, Mori S, van Zijl P, Bassett SS, Ross CA (2005) Regional white matter change in pre-symptomatic Huntington's disease: a diffusion tensor imaging study. Psychiatry Res 140(1):55–62

Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB (1988) Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci USA 85 (15):5733–5737

Reiner A, Del Mar N, Deng YP, Meade CA, Sun Z, Goldowitz D (2007) R6/2 neurons with intranuclear inclusions survive for prolonged periods in the brains of chimeric mice. J Comp Neurol 505(6):603–629

Reiner A, Shelby E, Wang H, Demarch Z, Deng Y, Guley NH, Hogg V, Roxburgh R, Tippett LJ, Waldvogel HJ, Faull RL (2013) Striatal parvalbuminergic neurons are lost in Huntington's disease: implications for dystonia. Mov Disord 28(12):1691–1699

Reisine TD, Fields JZ, Bird ED, Spokes E, Yamamura HI (1978) Characterization of brain dopaminergic receptors in Huntington's disease. Commun Psychopharmacol 2(2):79–84

Ribchester RR, Thomson D, Wood NI, Hinks T, Gillingwater TH, Wishart TM, Court FA, Morton AJ (2004) Progressive abnormalities in skeletal muscle and neuromuscular junctions of transgenic mice expressing the Huntington's disease mutation. Eur J Neurosci 20(11):3092–3114

Richardson EP Jr (1990) Huntington's disease: some recent neuropathological studies. Neuropathol Appl Neurobiol 16(6):451–460

Richfield EK, Herkenham M (1994) Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. Ann Neurol 36(4):577–584

Richfield EK, O'Brien CF, Eskin T, Shoulson I (1991) Heterogeneous dopamine receptor changes in early and late Huntington's disease. Neurosci Lett 132(1):121–126

Rodda RA (1981) Cerebellar atrophy in Huntington's disease. J Neurol Sci 50(1):147–157

Rodriguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ (2005) Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Mol Ther 12(4):618–633

Roizin L, Kaufman MA, Willson N, Stellar S, Liu JC (1976) Neuropathologic observations in Huntington's chorea. In: Zimmerman HM (ed) Progress in neuropathology, vol 3. Grune and Stratton, New York, pp 447–488

Roizin L, Stellar S, Liu JC (1979) Neuronal nuclear and cytoplasmic changes in Huntington's chorea: Electron microscope investigations. In: Chase TN, Wexler NS, Barbeau A (eds) Huntington's disease. Advances in neurology, vol 23. Raven Press, New York, pp 95–122

Roos RA, Pruyt JF, de Vries J, Bots GT (1985) Neuronal distribution in the putamen in Huntington's disease. J Neurol Neurosurg Psychiatry 48(5):422–425

Rosas HD, Hevelone ND, Zaleta AK, Greve DN, Salat DH, Fischl B (2005) Regional cortical thinning in preclinical Huntington disease and its relationship to cognition. Neurology 65 (5):745–747

Rosas HD, Koroshetz WJ, Chen YI, Skeuse C, Vangel M, Cudkowicz ME, Caplan K, Marek K, Seidman LJ, Makris N, Jenkins BG, Goldstein JM (2003) Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. Neurology 60 (10):1615–1620

Rosas HD, Lee SY, Bender AC, Zaleta AK, Vangel M, Yu P, Fischl B, Pappu V, Onorato C, Cha JH, Salat DH, Hersch SM (2010) Altered white matter microstructure in the corpus callosum in Huntington's disease: implications for cortical "disconnection". Neuroimage 49(4):2995–3004

Rosas HD, Liu AK, Hersch S, Glessner M, Ferrante RJ, Salat DH, van der Kouwe A, Jenkins BG, Dale AM, Fischl B (2002) Regional and progressive thinning of the cortical ribbon in Huntington's disease. Neurology 58(5):695–701

Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, Greve D, Hevelone ND, Hersch SM (2008) Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain 131(Pt 4):1057–1068

Rosas HD, Tuch DS, Hevelone ND, Zaleta AK, Vangel M, Hersch SM, Salat DH (2006) Diffusion tensor imaging in presymptomatic and early Huntington's disease: Selective white matter pathology and its relationship to clinical measures. Mov Disord 21(9):1317–1325

Ross CA, Poirier MA (2004) Protein aggregation and neurodegenerative disease. Nat Med 10 (Suppl):S10–17

Rüb U, Hoche F, Brunt ER, Heinsen H, Seidel K, Del Turco D, Paulson HL, Bohl J, von Gall C, Vonsattel JP, Korf HW, den Dunnen WF (2013) Degeneration of the cerebellum in Huntington's disease (HD): possible relevance for the clinical picture and potential gateway to pathological mechanisms of the disease process. Brain Pathol 23(2):165–177

Ruocco HH, Bonilha L, Li LM, Lopes-Cendes I, Cendes F (2008) Longitudinal analysis of regional grey matter loss in Huntington disease: effects of the length of the expanded CAG repeat. J Neurol Neurosurg Psychiatry 79(2):130–135

Sadikot AF, Parent A, Smith Y, Bolam JP (1992) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a light and electron microscopic study of the thalamostriatal projection in relation to striatal heterogeneity. J Comp Neurol 320 (2):228–242

Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, Bhide PG, Vonsattel JP, DiFiglia M (2001) Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol 60(2):161–172

Sapp E, Penney J, Young A, Aronin N, Vonsattel JP, DiFiglia M (1999) Axonal transport of N-terminal huntingtin suggests early pathology of corticostriatal projections in Huntington disease. J Neuropathol Exp Neurol 58(2):165–173

Sapp E, Schwarz C, Chase K, Bhide PG, Young AB, Penney J, Vonsattel JP, Aronin N, DiFiglia M (1997) Huntingtin localization in brains of normal and Huntington's disease patients. Ann Neurol 42(4):604–612

Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95 (1):55–66

Schiefer J, Landwehrmeyer GB, Luesse HG, Sprunken A, Puls C, Milkereit A, Milkereit E, Kosinski CM (2002) Riluzole prolongs survival time and alters nuclear inclusion formation in a transgenic mouse model of Huntington's disease. Mov Disord 17(4):748–757

Schroeder K (1931) Zur Klinik und Pathologie der Huntingtonschen Krankheit. J Psychol Neurol 43:183–201

Selemon LD, Rajkowska G, Goldman-Rakic PS (2004) Evidence for progression in frontal cortical pathology in late-stage Huntington's disease. J Comp Neurol 468(2):190–204

Seto-Ohshima A, Emson PC, Lawson E, Mountjoy CQ, Carrasco LH (1988) Loss of matrix calcium-binding protein-containing neurons in Huntington's disease. Lancet 1(8597):1252–1255

Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, Li XJ (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171(6):1001–1012

Sieradzan KA, Mann DM (2001) The selective vulnerability of nerve cells in Huntington's disease. Neuropathol Appl Neurobiol 27(1):1–21

Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G (2007) Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. Glia 55 (10):1074–1084

Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86(2):353–387

Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27(9):520–527

Sotrel A, Paskevich PA, Kiely DK, Bird ED, Williams RS, Myers RH (1991) Morphometric analysis of the prefrontal cortex in Huntington's disease. Neurology 41(7):1117–1123

Sotrel A, Williams RS, Kaufmann WE, Myers RH (1993) Evidence for neuronal degeneration and dendritic plasticity in cortical pyramidal neurons of Huntington's disease: a quantitative Golgi study. Neurology 43(10):2088–2096

Spampanato J, Gu X, Yang XW, Mody I (2008) Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. Neuroscience 157 (3):606–620

Spargo E, Everall IP, Lantos PL (1993) Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. J Neurol Neurosurg Psychiatry 56(5):487–491

Spielmeyer W (1926) Die anatomische Krankheitsforschung am Beispiel einer Huntingtonschen Chorea mit Wilsonschem Symptomenbild. Zeitschrift für die gesamte Neurologie und Psychiatrie (Berlin) 101:701–728

Spokes EG (1980) Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain 103(1):179–210

Strand AD, Baquet ZC, Aragaki AK, Holmans PA, Yang L, Cleren C, Beal MF, Jones L, Kooperberg C, Olson JM, Jones KR (2007) Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. J Neurosci 27(43):11758–11768

Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, Landwehrmeyer GB, Fox NC, Johnson H, Hicks SL, Kennard C, Craufurd D, Frost C, Langbehn DR, Reilmann R, Stout JC (2011) Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. Lancet Neurol 10(1):31–42

Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, Piccini P (2007) Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 130(Pt 7):1759–1766

Tattersfield AS, Croon RJ, Liu YW, Kells AP, Faull RLM, Connor B (2004) Neurogenesis in the striatum of the quinolinic acid lesion model of Huntington's disease. Neuroscience 127 (2):319–332

Telenius H, Kremer B, Goldberg YP, Theilmann J, Andrew SE, Zeisler J, Adam S, Greenberg C, Ives EJ, Clarke LA et al (1994) Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. Nat Genet 6(4):409–414

Tellez-Nagel I, Johnson AB, Terry RD (1974) Studies on brain biopsies of patients with Huntington's chorea. J Neuropathol Exp Neurol 33(2):308–332

Terplan K (1924) Zur pathologischen Anatomie der chronischen progressiven Chorea. Virchow's Arch f Pathol Anat (Berl) 252:146–176

Thompson-Vest NM, Waldvogel HJ, Rees MI, Faull RL (2003) GABA(A) receptor subunit and gephyrin protein changes differ in the globus pallidus in Huntington's diseased brain. Brain Res 994(2):265–270

Thompson JC, Snowden JS, Craufurd D, Neary D (2002) Behavior in Huntington's disease: dissociating cognition-based and mood-based changes. J Neuropsychiatry Clin Neurosci 14 (1):37–43

Thu DCV, Oorschot DE, Tippett LJ, Nana AL, Hogg VM, Synek BJ, Luthi-Carter R, Waldvogel HJ, Faull RLM (2010) Cell loss in the motor and cingulate cortex correlates with symptomatology in Huntington's disease. Brain 133(Pt 4):1094–1110

Timmers HJ, Swaab DF, van de Nes JA, Kremer HP (1996) Somatostatin 1-12 immunoreactivity is decreased in the hypothalamic lateral tuberal nucleus of Huntington's disease patients. Brain Res 728(2):141–148

Tippett LJ, Waldvogel HJ, Thomas SJ, Hogg VM, van Roon-Mom W, Synek BJ, Graybiel AM, Faull RLM (2007) Striosomes and mood dysfunction in Huntington's disease. Brain 130(Pt 1):206–221

Tokuno H, Chiken S, Kametani K, Moriizumi T (2002) Efferent projections from the striatal patch compartment: anterograde degeneration after selective ablation of neurons expressing mu-opioid receptor in rats. Neurosci Lett 332(1):5–8

Trifiletti RR, Snowman AM, Whitehouse PJ, Marcus KA, Snyder SH (1987) Huntington's disease: increased number and altered regulation of benzodiazepine receptor complexes in frontal cerebral cortex. Neurology 37(6):916–922

Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, Saudou F, Weber C, David G, Tora L et al (1995) Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. Nature 378(6555):403–406

Van der Burg JM, Bjorkqvist M, Brundin P (2009) Beyond the brain: widespread pathology in Huntington's disease. Lancet Neurol 8(8):765–774

Van Raamsdonk JM, Murphy Z, Selva DM, Hamidizadeh R, Pearson J, Petersen A, Bjorkqvist M, Muir C, Mackenzie IR, Hammond GL, Vogl AW, Hayden MR, Leavitt BR (2007) Testicular degeneration in Huntington disease. Neurobiol Dis 26(3):512–520

Van Roon-Mom WM, Hogg VM, Tippett LJ, Faull RLM (2006) Aggregate distribution in frontal and motor cortex in Huntington's disease brain. Neuroreport 17(6):667–670

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463(7284):1035–1041

Vogt C, Vogt O (1951) Precipitating and modifying agents in chorea. J Nerv Ment Dis 116:601–607

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57(5):369–384

Vonsattel JP, Keller C, Cortes Ramirez EP (2011) Huntington's disease—neuropathology. Handb Clin Neurol 100:83–100

Vonsattel JP, Keller C, Del Pilar AM (2008) Neuropathology of Huntington's disease. Handb Clin Neurol 89:599–618

Vonsattel JP, Myers RH, Bird ED, Ge P, Richardson EP Jr (1992) Huntington disease: 7 cases with relatively preserved neostriatal islets. Rev Neurol (Paris) 148(2):107–116

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44 (6):559–577

Voorn P, Gerfen CR, Groenewegen HJ (1989) Compartmental organization of the ventral striatum of the rat: immunohistochemical distribution of enkephalin, substance P, dopamine, and calcium-binding protein. J Comp Neurol 289(2):189–201

Waldvogel HJ, Faull RLM (1993) Compartmentalization of parvalbumin immunoreactivity in the human striatum. Brain Res 610(2):311–316

Waldvogel HJ, Kubota Y, Fritschy J, Mohler H, Faull RLM (1999) Regional and cellular localisation of GABA(A) receptor subunits in the human basal ganglia: An autoradiographic and immunohistochemical study. J Comp Neurol 415(3):313–340

Waters CM, Peck R, Rossor M, Reynolds GP, Hunt SP (1988) Immunocytochemical studies on the basal ganglia and substantia nigra in Parkinson's disease and Huntington's chorea. Neuroscience 25(2):419–438

Watkins LH, Rogers RD, Lawrence AD, Sahakian BJ, Rosser AE, Robbins TW (2000) Impaired planning but intact decision making in early Huntington's disease: implications for specific fronto-striatal pathology. Neuropsychologia 38(8):1112–1125

Weeks RA, Piccini P, Harding AE, Brooks DJ (1996) Striatal D1 and D2 dopamine receptor loss in asymptomatic mutation carriers of Huntington's disease. Ann Neurol 40(1):49–54

Weiss A, Roscic A, Paganetti P (2009) Inducible mutant huntingtin expression in HN10 cells reproduces Huntington's disease-like neuronal dysfunction. Mol Neurodegener 4:11

Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, Marder K, Penchaszadeh G, Roberts SA, Gayan J, Brocklebank D, Cherny SS, Cardon LR, Gray J, Dlouhy SR, Wiktorski S, Hodes ME, Conneally PM, Penney JB, Gusella J, Cha JH, Irizarry M, Rosas D, Hersch S, Hollingsworth Z, MacDonald M, Young AB, Andresen JM, Housman DE, De Young MM, Bonilla E, Stillings T, Negrette A, Snodgrass SR, Martinez-Jaurrieta MD, Ramos-Arroyo MA, Bickham J, Ramos JS, Marshall F, Shoulson I, Rey GJ, Feigin A, Arnheim N, Acevedo-Cruz A, Acosta L, Alvir J, Fischbeck K, Thompson LM, Young A, Dure L, O'Brien CJ, Paulsen J, Brickman A, Krch D, Peery S, Hogarth P, Higgins DS Jr, Landwehrmeyer B (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 101(10):3498–3503

White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL, MacDonald ME (1997) Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17(4):404–410

Whitehouse PJ, Trifiletti RR, Jones BE, Folstein S, Price DL, Snyder SH, Kuhar MJ (1985) Neurotransmitter receptor alterations in Huntington's disease: autoradiographic and homogenate studies with special reference to benzodiazepine receptor complexes. Ann Neurol 18 (2):202–210

Witjes-Ane MN, Zwinderman AH, Tibben A, van Ommen GJ, Roos RA (2002) Behavioural complaints in participants who underwent predictive testing for Huntington's disease. J Med Genet 39(11):857–862

Yamamoto A, Lucas JJ, Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101(1):57–66

Yohrling GJ, Jiang GC, DeJohn MM, Miller DW, Young AB, Vrana KE, Cha JH (2003) Analysis of cellular, transgenic and human models of Huntington's disease reveals tyrosine hydroxylase alterations and substantia nigra neuropathology. Brain Res Mol Brain Res 119 (1):28–36

Young AB, Greenamyre JT, Hollingsworth Z, Albin R, D'Amato C, Shoulson I, Penney JB (1988) NMDA receptor losses in putamen from patients with Huntington's disease. Science 241 (4868):981–983

Yung KK, Smith AD, Levey AI, Bolam JP (1996) Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. Eur J Neurosci 8(5):861–869

Zappacosta B, Monza D, Meoni C, Austoni L, Soliveri P, Gellera C, Alberti R, Mantero M, Penati G, Caraceni T, Girotti F (1996) Psychiatric symptoms do not correlate with cognitive decline, motor symptoms, or CAG repeat length in Huntington's disease. Arch Neurol 53(6):493–497

Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, Efstratiadis A (1995) Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. Nat Genet 11(2):155–163

Zuccato C, Cattaneo E (2007) Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol 81(5–6):294–330

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293 (5529):493–498

Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D, Cattaneo E (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35(1):76–83

Neurobiology of Huntington's Disease

Rebecca A.G. De Souza and Blair R. Leavitt

Abstract Of the neurodegenerative diseases presented in this book, Huntington's disease (HD) stands as the archetypal autosomal dominantly inherited neurodegenerative disorder. Its occurrence through generations of affected families was noted long before the basic genetic underpinnings of hereditary diseases was understood. The early classification of HD as a distinct hereditary neurodegenerative disorder allowed the study of this disease to lead the way in the development of our understanding of the mechanisms of human genetic disorders. Following its clinical and pathologic characterization, the causative genetic mutation in HD was subsequently identified as a trinucleotide (CAG) repeat expansion in the huntingtin (*HTT*) gene, and consequently, the *HTT* gene and huntingtin protein have been studied in great detail. Despite this concentrated effort, there is still much about the function of huntingtin that still remains unknown. Presented in this chapter is an overview of the current knowledge on the normal function of huntingtin and some of the potential neurobiologic mechanisms by which the mutant *HTT* gene may mediate neurodegeneration in HD.

Keywords Huntington's disease · Neurodegeneration · Huntingtin · Disease mechanisms

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R.A.G. De Souza · B.R. Leavitt (✉)
Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics, Child and Family Research Institute, University of British Columbia, 950 West 28th Avenue, Room 2020, Vancouver, BC, V5Z 4H4, Canada
e-mail: bleavitt@cmmt.ubc.ca

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1 HD Introduction

1.1 Introduction

In 1872, George Huntington published a short yet concise report entitled "On Chorea" based on his study of a seemingly hereditary and discrete disorder found in several generations of an American family (Huntington 1872). It was described as a disease primarily affecting adult individuals, causing both motor and cognitive/psychiatric deficits. This clinical report so encapsulated the symptoms and hereditary nature of this particular disorder that the disease itself became named Huntington's Chorea after George Huntington and is now known as Huntington's disease (HD) today.

HD is a progressive neurological disorder with predominantly adult onset that occurs in all human populations (world wide prevalence of 2.71 per 100,000 individuals), but has the highest prevalence in individuals of European origin (Pringsheim et al. 2012). Due to the hereditary nature and strong penetrance of HD, this disease has become the embodiment of efforts aimed at treating adult-onset neurological disorders. This chapter will outline the main clinical, genetic, and neurological features of HD and review the current understanding of wild-type *HTT* gene function and the effects of the disease-causing mutation on this gene and their potential pathogenic role in this devastating neurodegenerative disease.

1.2 Clinical Features

Many of the clinical features first described by George Huntington in "On Chorea" remain part of the clinical diagnosis used today, and primary of these are the movement disorders associated with HD with chorea being the defining feature.

Chorea is described as abnormal "dance-like" movements of the limbs, trunk, or face. The movement abnormalities in HD affect both voluntary and involuntary motor functions, with motor disturbances often occurring in a sequential manner as disease progresses (Mahant et al. 2003). During the first stages of HD, the motor symptoms primarily affect involuntary movements with chorea, hypotonia, and hyper-reflexia being the motor symptoms most commonly observed. As the disease progresses, voluntary movements become affected with rigidity and bradykinesia being most prominent. Voluntary motor abnormalities occur later in disease progression than involuntary ones and contribute to the majority of functional disability described by HD patients.

Cognitive deficits also follow a similar progression, generally beginning slowly at first and worsening as the disease progresses. Early in the disease process, patients often present with a slowing of intellectual processes, changes in personality, disinhibition, and reduced mental flexibility (Craufurd and Snowden 2002). As the disease worsens, there is a reduction in cognitive speed, flexibility, concentration, and patients progress toward "subcortical dementia" with aphasia and agnosia not commonly present. Neuropsychiatric symptoms are not uncommon in HD patients; however, they do not follow disease progression like the motor and cognitive deficits. These symptoms include depression, apathy, suicidal ideation, and anxiety (Craufurd and Snowden 2002). While the motor and cognitive symptoms described above are the major characteristics of HD and remain the focus of clinical diagnosis and treatment, other symptoms are also prevalent, and though they have historically received less attention, they still significantly impact patient's quality of life. These additional symptoms include sleep and circadian rhythm disorders, metabolic abnormalities primarily weight loss despite normal caloric intake, and testicular degeneration (Van Raamsdonk et al. 2007; Craufurd and Snowden 2002).

1.3 Neuropathology

The most striking neuropathologic feature of HD is the relatively selective and progressive degeneration of the caudate and putamen (collectively known as the striatum) (Vonsattel and DiFiglia 1998). This progressive shrinkage of the striatum correlates with disease progression and severity, with almost no striatal tissue remaining in very advanced stages of the disease. The neurodegeneration of the striatum is cell-type specific, with the medium spiny neurons (MSNs) being the neuronal subtype specifically affected in HD. The other major types of neurons in the striatum, the aspiny interneurons, are largely unaffected and relatively spared in HD. While other brain regions also show evidence of selective neurodegeneration in HD, none of them display degeneration to the extent seen in the striatum and most of degeneration outside of the striatum occurs later in disease (Hedreen et al. 1991; Spargo et al. 1993). These additional regions include the pyramidal projection neurons in layers V and VI of the cerebral cortex, the CA1 region of the

hippocampus, the globus pallidus, subthalamic nucleus, substantia nigra, cerebellum, and thalamus. Ubiquitinated HTT protein inclusions or aggregates are also a key neuropathologic feature of HD that were first described in mouse models of HD and were then later observed in HD patients (Davies et al. 1997).

1.4 Genetics

Despite the early characterization of HD as a well-defined clinical entity and subsequent studies that clearly established HD as an autosomal dominant disorder, the discovery of the causative gene did not occur until 1993 (The Huntington's Disease Collaborative Research Group 1993). This was due in part to the novel nature (at the time) of the HD mutation, a trinucleotide repeat expansion of the nucleotides cytosine, adenine, and guanine (a CAG repeat expansion). The discovery of the gene responsible for HD required large HD families to be identified and for novel molecular techniques capable of resolving the CAG expansion to be developed. Since its discovery in 1993, we have gained a better, though by no means comprehensive, understanding of the Huntington's disease gene, renamed the huntingtin gene (*HTT*) from its original designation of *IT15* (standing for *Interesting Transcript 15*). The CAG repeat expansion is a dynamic mutation, meaning that the length of the CAG expansion on a single allele can expand or contract as it is transmitted between generations and can vary between the cells of a single individual (Gonitel et al. 2008). Expansions of greater than 35 CAG repeats can result in Huntington's disease, and there is also a threshold of at least 39 CAG repeats after which the penetrance of HD is almost 100 %. In the following sections, we will discuss the main features of the *HTT* gene and protein and how these features are affected in the presence of a pathogenic CAG expansion.

2 Normal Huntingtin Function

2.1 HTT *Gene*

The identification of the *HTT* gene as the mutated gene in HD in 1993 has led to 20 years of intensive study into both the wild-type and mutated functions of the huntingtin protein (HTT). Despite this, there is much about the function of this protein that remains unknown. This section describes our current understanding of the wild-type function of the HTT protein. The *HTT* gene is found on chromosome 4 and is comprised of 67 exons that are translated to form a 348-kDa protein. While the HTT gene itself can be found in both invertebrates and vertebrates, it is most highly conserved among vertebrates (80 %) (Baxendale et al. 1995; Gissi et al. 2006; Tartari et al. 2008). The protein sequence of HTT, however, bears little

overall homology to any other known protein making inferences about the function of the HTT protein through comparisons of related proteins difficult, though not impossible.

2.2 Huntingtin Protein Sequence

Through computational analyses, searching for similarities to known protein domains, HTT has been found to contain 37 putative HEAT domains that are thought to be involved in protein–protein interactions (Andrade and Bork 1995; Takano and Gusella 2002). These 37 HEAT domains are conserved throughout vertebrates and suggest that the protein–protein interactions they dictate are similar across vertebrates. Shortly upstream of the HEAT repeats is another region conserved only in higher vertebrates designated as the polyproline stretch (Steffan et al. 2004). This short repeat of proline amino acids is thought to be important in the folding of the HTT protein and may function to keep the protein soluble. Sequence analysis has also revealed a fully functional and active C-terminal nuclear export signal and a less active nuclear localization signal (Xia et al. 2003). The presence of the nuclear export signal and that of the multiple HEAT protein–protein domains suggest that HTT may be involved in transportation of molecules from the nucleus.

2.3 Posttranslational Modification of Huntingtin

Consistent with the role for HTT in molecular transport hypothesized by its HEAT domains are some of its posttranslational modifications, namely palmitoylation (Young et al. 2012). Palmitoylated proteins are often involved in the assembly of vesicle trafficking control complexes and assembly of synaptic vesicle function complexes (Huang et al. 2004). HTT is palmitoylated by huntingtin-interacting protein 14 (HIP14). In addition to palmitoylation, HTT is also sumoylated and ubiquitinated at N-terminal lysines K6, K9, and K15 (Kalchman et al. 1996; Steffan et al. 2004). The proteins that sumoylate or ubiquintinate these sites compete for modification, with ubiquitination being a marker for degradation of the HTT protein through the ubiquitin–ligase degradation pathway and sumoylation preventing this degradation (Ehrnhoefer et al. 2011). HTT is also phosphorylated at serines 421 and 434, and this phosphorylation influences the cellular localization, function, and cleavage of the HTT protein, discussed below (Humbert et al. 2002; Luo et al. 2005; Warby et al. 2005).

2.4 Cleavage of the Huntingtin Protein

The HTT protein contains three protease cleavage consensus sites that generate shorter fragments from the full-length HTT protein (Goldberg et al. 1996; Wellington et al. 1998). In addition to these three sites, there are an additional three caspase cleavage sites and two calpain cleavage sites N-terminal to the primary caspase cleavage sites. Both the wild-type and mutant forms of the HTT protein can be cleaved (more on mutant cleavage in the following section), producing fragments of varying length, function, and cellular localization. Brain region-specific cleavage of HTT protein has also been described, suggesting that different fragment lengths may have cell-type-specific functions adding additional complexity to the function of this protein (Mende-Mueller et al. 2001).

2.5 Regional Expression of Huntingtin

Despite the association of mutant huntingtin with selective neurodegeneration in specific brain regions, HTT is actually ubiquitously expressed, at low levels, throughout the body (Van Raamsdonk et al. 2007), the expression in the brain and testis being generally about ~5-fold greater than that in other peripheral tissues. The function of HTT outside of the CNS has not been studied extensively, and we can only hypothesize, based on HTT's potential role in neuronal survival (discussed below), that in peripheral cells, it may have a similar primary function. It is also unknown what cellular pathways drive this differential expression between the CNS and periphery. Studies into the composition and function of the *HTT* gene promoter have highlighted a few important regulatory regions and transcription factors within the proximal promoter; however, none of these have identified potential mechanisms for this differential expression (Lin et al. 1995; Holzmann et al. 2001; Tanaka et al. 2004; Feng et al. 2006; Wang et al. 2012). In terms of furthering our understanding of the function of this protein, two studies have found that HTT expression is in part regulated by the transcription factor p53, which is a well-known regulator of cell survival (Feng et al. 2006). This suggests that HTT may function in cell survival both within and outside the CNS. Once produced, the HTT gene transcript has two alternate transcripts, with the larger of the two being preferentially expressed in the brain (Lin et al. 1993). The functional relevance of these two transcripts remains unclear; however, in mutant condition, an additional short splice variant encompassing exon 1 has been recently identified and may play a role in HD pathogenesis (Sathasivam et al. 2013).

2.6 Developmental Function of Huntingtin

So far, we have discussed genetic and molecular features that have given us insights into the function of the HTT protein, and we now turn to functional studies based on these inferences to further explore the function of this protein. Starting with the inference that given HTT's high conservation across several phyla, we can assume that this is a very important protein and that loss of the *HTT* gene would have serious consequences for the organism. This has been shown to be true in mouse HTT "knockout" models (*Hdh nullizygous mice*) which are embryonic lethal (Nasir et al. 1995; Duyao et al. 1995; Zeitlin et al. 1995). Interestingly, embryonic lethality in these mice occurs before embryonic day 8.5. This is prior to gastrulation and formation of the nervous system, highlighting the role of HTT not only within the CNS, but in other peripheral tissues. In this case, it seems that complete loss of HTT within extra-embryonic tissues results in defects in organization of these tissues causing embryonic lethality (Leavitt et al. 2001; Van Raamsdonk et al. 2005a). Other studies have shown that a reduction in embryonic HTT, below 50 %, after gastrulation, while not lethal, leads to abnormalities in the formation of the epiblast (White et al. 1997). This is the structure that gives rise to the neural tube. These abnormalities in turn lead to a reduction in neurogenesis and abnormalities in the structure of the cortex and striatum. In addition to these studies, work with chimeric mice, where *Hdh* null stem cells are introduced into a wild-type blastocyst, revealed a region-specific need for HTT in both the cortex and striatum as these regions were devoid of *Hdh* null cells (Reiner et al. 2003). Combined, these studies into the requirement of HTT early in development highlight HTT's important function both outside of the CNS and within.

2.7 Huntingtin and Cellular Survival

The studies highlighted above demonstrate HTT's important role in actively dividing cells during development, but it also appears to play a role in post-mitotic cells of the adult brain. HD is an adult-onset disorder, and HTT is widely expressed in adult tissues, especially post-mitotic neuronal cells. It is important to understand the potential differences in HTT function between these different cell types. As described previously, HTT has been suggested to play a role in cellular survival. This proposed function has been studied in both in vitro and in vivo experiments. Using conditionally immortalized striatum-derived cells that stably overexpress wild-type human HTT, various toxic stimuli were applied. Overexpression of wild-type human HTT was able to protect against these toxic stimuli (Rigamonti et al. 2000). In vivo, it has been found that overexpression of wild-type HTT protects against ischemic and excitotoxic injury (Zhang et al. 2003; Leavitt et al. 2006). The mechanisms through which HTT conducts its pro-survival activities have been suggested to include prevention of processing of pro-caspase 9, as well as preventing the formation of the pro-apoptotic protein huntingtin-interacting protein 1 complex (Rigamonti et al. 2000, 2001).

One of the most convincing mechanisms for HTT's pro-survival role in the CNS is via HTT's regulation of brain-derived neurotrophic factor (BDNF), a neurotrophin that plays a critical role in the survival of striatal cells. BDNF is not produced in the stratum itself, but is expressed by pyramidal cells in the cerebral cortex and anterogradely transported along cortico-striatal afferents where it is released at axon terminals and taken up by striatal neurons (Mizuno et al. 1994; Ventimiglia et al. 1995; Altar et al. 1997). This interaction between cortical and striatal neurons allows the striatal neurons to be resistant to glutamate-mediated excitotoxic neurodegeneration (Bemelmans et al. 1999; Pineda et al. 2005). The HTT protein has also been shown to act directly on BDNF levels, with in vivo and in vitro data showing that overexpression of wild-type HTT results in an increase in BDNF levels and overexpression of mutant HTT results in a decrease in BDNF levels (Zuccato et al. 2001). Further analysis has shown that this increase is due to HTT acting directly on transcription of the BDNF through HTT's interaction and sequestration of RE1-silencing transcription factor (REST also known as neuronal restrictive silencing factor (NRSF)). REST/NRSF binds to a repressor element in the BDNF promoter responsible for generating the particular type of BDNF that is transported to the striatum (Zuccato et al. 2001, 2003). By sequestering REST/NRSF and not allowing it to interact with the BDNF promoter, HTT allows for increased transcription, and therefore translation, of BDNF. As the REST/NRSF transcription factor is not specific for the BDNF promoter, REST/NRSF response elements can be found in other promoters, and HTT may play a role in the regulation of other REST/NRSF genes.

2.8 Huntingtin and Vesicle Transport

As mentioned previously, HTT has been predicted to be involved in vesicular transport, and this has been found to be true both in the case of BDNF transport and in the case of other neurotransmitters. In cortical neurons that project to the striatum, wild-type HTT colocalizes with BDNF, suggesting that HTT may play a role in guiding BDNF toward striatal cells (Gauthier et al. 2004). In cultured cells, wild-type HTT increases the vesicular transport of BDNF along microtubles, but if HTT is knocked down using RNAi, this transport is slowed down. HTT has also been found to interact with the p150 (Glued) subunit of dynactin, a key component of the molecular motor which moves vesicles along microtubules (Imarisio et al. 2008). Through interactions with huntingtin-associated protein 1 (HAP1), Glued, and BDNF, HTT can increase the transport of BDNF along microtubles. More recently, HTT has been found to regulate the transport of the BDNF receptor TrkB in striatal neurons, further highlighting the role of HTT in BDNF function (Liot et al. 2013).

2.9 Huntingtin and the Synapse

The interaction between HTT and vesicles does not end once they have reached their intended cellular destination. Once HTT and its vesicular payload reach the cortical synapse, HTT appears to play additional roles in synaptic vesicle transmission. HTT interacts with several proteins involved for exocytosis and endocytosis at synaptic terminals such as HIP1, HIP14, HAP1, HAP40, PACSIN1, SH3Gl3, clatherin, and dynamin. A key synaptic transmission molecule that HTT interacts with is PSD-95, one of a family of proteins that binds the NMDA and Kainate receptors at the postsynaptic density (Sheng and Kim 2002). In addition to direct interaction with proteins at the synapse, HTT appears to regulate the expression of proteins that are involved with synapse function. Complexin II and rabphilin 3A are both proteins involved in the exocytosis of vesicles both of which display altered expression in mouse models and human cell lines of HD (Morton and Edwardson 2001; Smith et al. 2005). Finding additional roles for HTT at the synapse reinforces the concept of HTT as a positive regulator of neuronal cell survival and function.

3 Mutant Huntingtin

3.1 The Mutation

As previously discussed in the introduction, the causative mutation in HD is a CAG trinucleotide repeat expansion in the *HTT* gene. This CAG expansion is localized in exon 1 of the *HTT* gene; when the gene is translated into the HTT protein, the expansion results in an expanded polyglutamine stretch. The CAG expansion itself is a dynamic mutation, with the length of the CAG changing both between generations and within different cells in an individual. With longer expansions, there is a predisposition toward lengthening of the CAG repeat versus shortening (MacDonald et al. 1999; Djoussé et al. 2004; Chattopadhyay et al. 2005). HD patients have a *HTT* allele that contains at least 39 CAG repeats, and this is quite longer than the average 17–20 repeats found on most WT alleles (Warby et al. 2009). Patients with longer repeats, on average, have an earlier age of onset and a more severe phenotype. This inverse correlation between length and age of onset accounts for between 60 and 70 % of the variance in age of onset of HD patients (Andrew et al. 1993).

The inverse correlation between CAG repeat length and age of onset and the dynamic nature of this mutation combine to create the phenomenon of anticipation in HD families. Anticipation is defined as the phenomenon where subsequent generations of family members experience earlier ages of onset or more severe disease than their ancestors. Anticipation is also a concern for individuals carrying so-called intermediate alleles, 27–35 CAGs. It has been found that a specific genetic haplotype is found on 95 % of HD alleles and this same haplotype is overrepresented on intermediate alleles compared to wild-type alleles (Andrew et al. 1993).

This suggests that there may be predisposing factors present in this haplotype that increase the instability of the CAG repeat. As the CAG lengths on these intermediate alleles further expand/contract as they traverse generations, it is expected that they will eventually lead to fully expanded HD alleles and new proband patients and families.

3.2 Mutant Huntingtin Toxicity

3.2.1 Gain of Function and Loss of Function

Given that the CAG repeat expansion results in an expanded polyglutamine tract which affects the conformation of the HTT protein, it is important to understand how the altered polyglutamine tract affects the function of the protein. As we will discuss in the following sections, the HD mutation does not appear to be a complete loss of function but is primarily a novel toxic gain of function mutation, although elements of both appear to play a role in HD pathogenesis. It is interesting to note that while the mutation may affect certain aspects of wild-type HTT function, the mutant protein appears to retain much of the basic function of the wild-type protein. This is best displayed in Hdh knockout mice that, as mentioned above, are embryonic lethal; however, if mutant HTT is added back to these mice, they survive and their early development remains normal (Van Raamsdonk et al. 2005a).

In addition, individuals who are homozygous for the mutant allele develop normally and have no apparent defects until later on in life (Wexler et al. 1987; Myers et al. 1989). It appears, at least early in development, that mutant HTT performs all the functions of wild-type HTT, and it is only later that the CAG expansion may interfere with wild-type function. As discussed in the previous section, postnatal removal of wild-type HTT in mature neurons results in cellular death, and mutant HTT also causes similar cellular death, indicating that perhaps loss of the wild-type function due to the CAG expansion is having a similar effect. It is likely that the combination of loss of wild-type HTT function as well as new toxic gain of function by mutant HTT that results in the phenotype seen in HD.

3.2.2 Protein Aggregation

Before furthering our discussion of the effect, the CAG expansion has on the HTT protein the issue of HTT inclusions or aggregates must be addressed. Protein aggregates that are visible on pathologic examination were first described in mouse models of HD and were then later observed in HD patients (Davies et al. 1997). These aggregates are made up of insoluble proteins, of which HTT is a main component—however, other proteins have been found in the aggregates as well. It was only after the identification of these aggregates in mouse models that they were identified in HD patient brain tissues as well. The presence of these misfolded

protein aggregates places HD within the family of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) as diseases that have a proteinopathy as their putative pathologic basis. It was initially believed that these insoluble protein aggregates were directly pathogenic and contributed to the neurodegeneration seen in HD. This view has changed as it has been found that the cells containing these aggregates are not necessarily the ones that are dying. In fact, it appears that cells that do not form aggregates are more sensitive to neurotoxicity (Gauthier et al. 2004). This suggests that the aggregates may in fact be a natural compensatory mechanism that protects neurons from degeneration. The regional expression of huntingtin and the pattern and timing of inclusion formation do not correlate with selective neurodegeneration in HD and are not thought to be primary determinants of pathology. In addition, not all mouse models of HD demonstrate aggregate formation concurrent with neuronal cell loss, further providing evidence that the insoluble aggregates seen in HD are not necessarily causative of disease (Arrasate et al. 2004; Van Raamsdonk et al. 2005b).

3.2.3 Cleavage of Mutant HTT

As discussed in the wild-type section, the HTT protein is cleaved at several sites, generating fragments of different lengths. Mutant HTT is also cleaved, although in addition to the wild-type cleavage sites, it appears that mutant HTT is further cleaved and generates additional fragments. These additional fragments are thought to be the toxic species and contribute to disease pathogenesis. Reducing the activity of caspases and calpains reduces the generation of these additional fragments and in turn delays disease progression (Gafni and Ellerby 2002; Gafni et al. 2004). In addition to altered cleavage of the HTT protein, it has been found that alternate splicing of the mutant HTT allele also produces a short exon 1 mRNA that is later translated into a exon 1 fragment (Mende-Mueller et al. 2001; Sathasivam et al. 2013).

3.2.4 Mutant HTT and BDNF

As discussed in the previous section, HTT plays a role in the regulation and transport of BDNF. It is therefore unsurprising to find that the mutant CAG expansion alters the relationship between HTT and BNDF. The CAG expansion prevents HTT from stimulating BDNF transcription in cortical neurons. This is due to mutant HTT's inability to sequester REST/NRSF in the cytoplasm, which allows it to translocate to the nucleus and repress BDNF transcription (Zuccato et al. 2001, 2003). Mutant HTT also represses BDNF vesicular trafficking along microtubules, resulting in less BDNF being transported from the cortex to the striatum (Gauthier et al. 2004). This reduction in the amount of BDNF reaching the striatum may account for the selective degeneration of striatal neurons seen in HD. This hypothesis is supported by evidence from mice with conditional knockout of BDNF in the cortex (Baquet et al. 2004). These mice have decreased cortical and striatal

volumes and morphological differences in MSN numbers. In addition, using an exon 1 fragment mouse model of HD, R6/1, in combination with only one functional BDNF allele caused an earlier onset of phenotype and enhancement of motor abnormalities compared to R6/1 mice with two functional BDNF alleles (Canals et al. 2004; Pineda et al. 2005).

3.2.5 Transcriptional Dysregulation

As was mentioned in the wild-type function section of this chapter, HTT regulates BDNF transcription through its interaction with the transcriptional repressor REST. Given this known interaction with a transcriptional regulator, it is unsurprising to find that in its mutated form, HTT disrupts the normal expression of not only BDNF but a multitude of other genes regulated by REST including non-coding RNAs (Zuccato et al. 2007; Johnson et al. 2008). The transcriptional dysregulation seen in HD is not localized to REST-regulated genes alone. The conformational changes conferred by the CAG repeat result in mutant HTT abnormally interacting with several other transcription factors, resulting in widespread transcriptional changes. These transcription factors include TATA-binding protein/TFIID, TAFII130 (a coactivator in CREB-dependent transcription), SP1, p53, and nuclear receptor corepressor (NCoR) (Boutell et al. 1999; Steffan et al. 2000; Shimohata et al. 2000; Dunah et al. 2002). Mutant HTT further affects transcriptional regulation by interacting with CBP and the p300/CBP-associated factor P/CAF which are involved in chromatin remodeling through posttranslational modification of histones (Boutell et al. 1999; Steffan et al. 2000). In total, more than 81 % of striatal-enriched genes are downregulated in both mouse models and human HD caudate samples, signifying the impact this dysregulation has on disease pathogenesis (Desplats et al. 2006).

3.2.6 Mitochondrial Dysfunction

As mentioned briefly in the clinical features section of this chapter, HD patients experience metabolic abnormalities and evidence for energetic dysfunction. A main contributing factor to these symptoms is mutant HTT's effect on mitochondria. Mutant HTT has been found to increase mitochondrial fragmentation, disrupt the biogenesis and trafficking of mitochondria, and lower mitochondrial membrane potential (Panov et al. 2003; Chang et al. 2006; Milakovic et al. 2006; Wang et al. 2009). ATP production by mitochondria has also been found to be altered in the presence of mutant HTT via reduced expression of oxidative phosphorylation enzymes that mediate the production of ATP (Gu et al. 1996; Benchoua et al. 2006). In line with the transcriptional dysregulation discussed above, mutant HTT's abnormal interaction with transcription factors required for CREB-dependent transcription factors results in the downregulation of PGC1-a, a well-characterized regulator of mitochondrial biogenesis (Cui et al. 2006). Mutant HTT also prevents

mitochondria from regulating cellular calcium homeostasis. This results in inefficient respiration, lowered levels of calcium capacity in mitochondria, and sensitivity to increases in calcium resulting from glutamate excitotoxicity as discussed below (Milakovic et al. 2006; Fernandes et al. 2007).

3.2.7 Excitotoxicity in HD

Excitotoxicity specifically refers to neuronal death induced via overstimulation by excitatory neurotransmitters and is proposed to be a major contributor to the selective neurodegeneration of MSNs in HD. MSNs primarily receive excitatory glutamatergic input from the cortex and are particularly sensitive to toxicity induced from the glutamate analogues quinolinic and kainic acid (McGeer and McGeer 1976; Schwarcz et al. 1984). Glutamate activates receptors including the NMDA receptor, which transports calcium into the neuron. Mutant HTT disrupts mitochondrial calcium homeostasis, making neurons more sensitive to changes in calcium levels including calcium influx from NMDA receptor stimulation. NMDA receptors are composed of two subunits, one of which, NR2, has two main subtypes, A and B, that react differently to the presence of mutant HTT. NMDA receptors containing NR2B influx more calcium in the presence of mutant HTT when stimulated, and this subtype can be found in tissues with higher vulnerability to mutant HTT (Li et al. 2003, 2004). The subcellular localization and posttranslational modification of NMDA receptors are also known to alter the specific type of response induced by stimulation. NMDA receptors are located both in the synaptic and in the extra-synaptic plasma membranes, and this localization is specified through posttranslational modification. Cleavage of the C-terminus by calpain and de-phosphorylation of NMDA receptor subunits by the phosphatase STEP result in reduced NMDA receptor localization to the synaptic membrane (Gladding et al. 2012). Both calpain and STEP are expressed at higher levels in the presence of mutant HTT and may explain the higher frequency of extra-synaptic NMDA receptors seen in HD mouse models (Cowan et al. 2008; Graham et al. 2009).

3.2.8 RNA Toxicity

The predominant view in the field of HD research is that the HD mutation confers a novel structural or functional change in the HTT protein and it is the change in the protein conformation that results in pathogenesis. The prevailing view of HD pathogenesis may be challenged by recent findings implicating a potential role of mutant RNA transcripts in mutant HTT toxicity. The majority of our understanding of RNA toxicity comes from studying the CTG expansion in myotonic dystrophy type 1 (DM1) (Fiszer and Krzyzosiak 2013). In DM1, the CTG expansion in patients ranges from 50 to 3,000 repeats present in the untranslated region of the dystrophia myotonica protein kinase gene and it is the presence of this repeat in the mRNA transcript of the mutated allele that causes pathogenesis. While the

threshold of repeats differs greatly between HD and DM1, with DM1 being higher, there remains a significant proportion of HD alleles with repeat sizes within the pathogenic range seen in DM1. In addition, analysis of the hairpin structures generated by CAG and CUG (remember that during transcription, Ts are transcribed as Us, converting a CTG repeat into a CUG repeat) repeats is similar (Jasinska et al. 2003; Sobczak et al. 2003). In both cases, CAG and CUG repeats of normal length form small and unstable hairpins and larger repeats form longer and more stable ones, respectively.

By comparing RNA molecules carrying either pure CAG repeats or CAG repeats interrupted by CAA codons (both codons translate to glutamine, but the CAA codon is unable to form hairpins) in Drosophila, it was found that the CAA-interrupted molecules produced less neurodegenerative features (Sobczak et al. 2003). In addition, studies looking at expanded CAG repeats in human HeLa and SK-N-MC cells showed formation of nuclear RNA foci and alternative splicing, hallmarks found in DM1 (Mykowska et al. 2011). HTT transcripts have also been found in RNA foci in human HD fibroblasts (Fiszer and Krzyzosiak 2013). Aberrant splicing of the *HTT* gene has also been attributed to the CAG expansion using a similar mechanism of RNA toxic gain of function seen in DM1 (Sathasivam et al. 2013).

RNAi mechanisms have also been implicated in HD, with studies in drosophila showing that coexpression of expanded complementary CAG and CUG repeats results in dsRNA that is cleaved by the Dicer-2 pathway forming CAG/CUG siRNA that causes neurodegeneration (Yu et al. 2011; Lawlor et al. 2011). This finding is collaborated by studies on the antisense HTT transcript, which contains a CUG repeat and has been shown to regulate sense HTT transcription in a repeat-dependent manner and is dependent on the Dicer and RISC pathways (Chung et al. 2011). In addition to this evidence, it has been shown in human cell lines that expanded CAG repeats flanked by the HTT exon 1 sequence generate small RNA species through Dicer and cause a downstream silencing of genes through Ago-2 (Bañez-Coronel et al. 2012). These effects were only seen in CAG repeats and not in CAA repeats.

The potential contribution of RNA toxicity to HD disease pathogenesis is a new area of research in the field of HD, and it will take time to develop the necessary tools to study the potential role of RNA toxicity in vivo.

4 Concluding Remarks

In this chapter, we have endeavored to give the reader an overview of the clinical features of HD, an appreciation of our current knowledge of the *HTT* gene and protein function, and finally a perspective on how the CAG repeat expansion mutation in the *HTT* gene causes HD. As with any review, it is impossible to cover every aspect of the current literature and there is important research that has not been covered here due to restrictions of space. We have highlighted the complex

structure and posttranslational processing of the HTT protein and outlined multiple proposed functions of the wild-type HTT protein. The potential neurotoxic effects of the CAG mutation in HD, how this may affect the HTT protein or mRNA, and how this may contribute to disease pathogenesis were reviewed.

The scientific community has known of the *HTT* gene and the CAG mutation that causes HD for over 20 years now, and it is understandable to expect that treatments and cures for HD should be progressing quickly—if not already available. Unfortunately, given the complexity and uniqueness of the HTT protein, which we have attempted to highlight throughout this chapter, this is not the case. There are currently no approved treatments aimed at reducing or eliminating HD pathogenesis. Current treatments for HD consist of drugs aimed at alleviating symptoms of the disease. This shortage of treatment options is not due to lack of effort from the scientific community, but due to the unique challenges that HD presents. We hope that the reader comes away from this chapter with a greater appreciation of the complexities of the *HTT* gene, for the diverse functions of the huntingtin protein, and the unique challenges facing researchers studying the effects of the HD mutation and developing novel treatment strategies for this devastating neurodegenerative disorder.

References

Altar CA, Cai N, Bliven T et al (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389:856–860. doi:10.1038/39885

Andrade MA, Bork P (1995) HEAT repeats in the Huntington's disease protein. Nat Genet 11:115–116. doi:10.1038/ng1095-115

Andrew SE, Goldberg YP, Kremer B et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4:398–403. doi:10.1038/ng0893-398

Arrasate M, Mitra S, Schweitzer ES et al (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810. doi:10.1038/nature02998

Bañez-Coronel M, Porta S, Kagerbauer B et al (2012) A pathogenic mechanism in Huntington's disease involves small CAG-repeated RNAs with neurotoxic activity. PLoS Genet 8:e1002481. doi:10.1371/journal.pgen.1002481

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250–4258. doi:10.1523/JNEUROSCI.3920-03.2004

Baxendale S, Abdulla S, Elgar G et al (1995) Comparative sequence analysis of the human and pufferfish Huntington's disease genes. Nat Genet 10:67–76. doi:10.1038/ng0595-67

Bemelmans AP, Horellou P, Pradier L et al (1999) Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, as demonstrated by adenoviral gene transfer. Hum Gene Ther 10:2987–2997. doi:10.1089/10430349950016393

Benchoua A, Trioulier Y, Zala D et al (2006) Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. Mol Biol Cell 17:1652–1663. doi:10.1091/mbc.E05-07-0607

Boutell JM, Thomas P, Neal JW et al (1999) Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. Hum Mol Genet 8:1647–1655

Canals JM, Pineda JR, Torres-Peraza JF et al (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 24:7727–7739. doi:10.1523/JNEUROSCI.1197-04.2004

Chang DTW, Rintoul GL, Pandipati S, Reynolds IJ (2006) Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. Neurobiol Dis 22:388–400. doi:10.1016/j.nbd.2005.12.007

Chattopadhyay B, Baksi K, Mukhopadhyay S, Bhattacharyya NP (2005) Modulation of age at onset of Huntington disease patients by variations in TP53 and human caspase activated DNase (hCAD) genes. Neurosci Lett 374:81–86. doi:10.1016/j.neulet.2004.10.018

Chung DW, Rudnicki DD, Yu L, Margolis RL (2011) A natural antisense transcript at the Huntington's disease repeat locus regulates HTT expression. Hum Mol Genet 20:3467–3477. doi:10.1093/hmg/ddr263

Cowan CM, Fan MMY, Fan J et al (2008) Polyglutamine-modulated striatal calpain activity in YAC transgenic huntington disease mouse model: impact on NMDA receptor function and toxicity. J Neurosci 28:12725–12735. doi:10.1523/JNEUROSCI.4619-08.2008

Craufurd D, Snowden JS (2002) Neuropsychological and neuropsychiatric aspects of Huntington's disease. In: Harper P, Bates G, Jones L (eds) Huntington's disease, 3rd edn. Oxford University Press, Oxford, pp 62–94

Cui L, Jeong H, Borovecki F et al (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 127:59–69. doi:10.1016/j.cell.2006.09.015

Davies SW, Turmaine M, Cozens BA et al (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548

Desplats PA, Kass KE, Gilmartin T et al (2006) Selective deficits in the expression of striatal-enriched mRNAs in Huntington's disease. J Neurochem 96:743–757. doi:10.1111/j.1471-4159.2005.03588.x

Djoussé L, Knowlton B, Hayden MR et al (2004) Evidence for a modifier of onset age in Huntington disease linked to the HD gene in 4p16. Neurogenetics 5:109–114. doi:10.1007/s10048-004-0175-2

Dunah AW, Jeong H, Griffin A et al (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 296:2238–2243. doi:10.1126/science.1072613

Duyao MP, Auerbach AB, Ryan A et al (1995) Inactivation of the mouse Huntington's disease gene homolog Hdh. Science 269:407–410

Ehrnhoefer DE, Sutton L, Hayden MR (2011) Small changes, big impact: posttranslational modifications and function of huntingtin in Huntington disease. Neuroscientist 17:475–492. doi:10.1177/1073858410390378

Feng Z, Jin S, Zupnick A et al (2006) p53 tumor suppressor protein regulates the levels of huntingtin gene expression. Oncogene 25:1–7. doi:10.1038/sj.onc.1209021

Fernandes HB, Baimbridge KG, Church J et al (2007) Mitochondrial sensitivity and altered calcium handling underlie enhanced NMDA-induced apoptosis in YAC128 model of Huntington's disease. J Neurosci 27:13614–13623. doi:10.1523/JNEUROSCI.3455-07.2007

Fiszer A, Krzyzosiak WJ (2013) RNA toxicity in polyglutamine disorders: concepts, models, and progress of research. J Mol Med 91:683–691. doi:10.1007/s00109-013-1016-2

Gafni J, Ellerby LM (2002) Calpain activation in Huntington's disease. J Neurosci 22:4842–4849

Gafni J, Hermel E, Young JE et al (2004) Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. J Biol Chem 279:20211–20220. doi:10.1074/jbc.M401267200

Gauthier LR, Charrin BC, Borrell-Pagès M et al (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118:127–138. doi:10.1016/j.cell.2004.06.018

Gissi C, Pesole G, Cattaneo E, Tartari M (2006) Huntingtin gene evolution in Chordata and its peculiar features in the ascidian Ciona genus. BMC Genomics 7:288. doi:10.1186/1471-2164-7-288

Gladding CM, Sepers MD, Xu J et al (2012) Calpain and STriatal-Enriched protein tyrosine phosphatase (STEP) activation contribute to extrasynaptic NMDA receptor localization in a Huntington's disease mouse model. Hum Mol Genet 21:3739–3752. doi:10.1093/hmg/dds154

Goldberg YP, Nicholson DW, Rasper DM et al (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. Nat Genet 13:442–449. doi:10.1038/ng0896-442

Gonitel R, Moffitt H, Sathasivam K et al (2008) DNA instability in postmitotic neurons. Proc Natl Acad Sci USA 105:3467–3472. doi:10.1073/pnas.0800048105

Graham RK, Pouladi MA, Joshi P et al (2009) Differential susceptibility to excitotoxic stress in YAC128 mouse models of Huntington disease between initiation and progression of disease. J Neurosci 29:2193–2204. doi:10.1523/JNEUROSCI.5473-08.2009

Gu M, Gash MT, Mann VM et al (1996) Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 39:385–389. doi:10.1002/ana.410390317

Hedreen JC, Peyser CE, Folstein SE, Ross CA (1991) Neuronal loss in layers V and VI of cerebral cortex in Huntington's disease. Neurosci Lett 133:257–261

Holzmann C, Schmidt T, Thiel G et al (2001) Functional characterization of the human Huntington's disease gene promoter. Brain Res Mol Brain Res 92:85–97

Huang K, Yanai A, Kang R et al (2004) Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. Neuron 44:977–986. doi:10.1016/j.neuron.2004.11.027

Humbert S, Bryson EA, Cordelières FP et al (2002) The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. Dev Cell 2:831–837

Huntington G (1872) On Chorea. Med Surg Rep 26:317–321

Imarisio S, Carmichael J, Korolchuk V et al (2008) Huntington's disease: from pathology and genetics to potential therapies. Biochem J 412:191–209. doi:10.1042/BJ20071619

Jasinska A, Michlewski G, de Mezer M et al (2003) Structures of trinucleotide repeats in human transcripts and their functional implications. Nucleic Acids Res 31:5463–5468

Johnson R, Zuccato C, Belyaev ND et al (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. Neurobiol Dis 29:438–445. doi:10.1016/j.nbd.2007.11.001

Kalchman MA, Graham RK, Xia G et al (1996) Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. J Biol Chem 271:19385–19394

Lawlor KT, O'Keefe LV, Samaraweera SE et al (2011) Double-stranded RNA is pathogenic in Drosophila models of expanded repeat neurodegenerative diseases. Hum Mol Genet 20:3757–3768. doi:10.1093/hmg/ddr292

Leavitt BR, Guttman JA, Hodgson JG et al (2001) Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo. Am J Hum Genet 68:313–324. doi:10.1086/318207

Leavitt BR, Van Raamsdonk JM, Shehadeh J et al (2006) Wild-type huntingtin protects neurons from excitotoxicity. J Neurochem 96:1121–1129. doi:10.1111/j.1471-4159.2005.03605.x

Li L, Fan M, Icton CD et al (2003) Role of NR2B-type NMDA receptors in selective neurodegeneration in Huntington disease. Neurobiol Aging 24:1113–1121

Li L, Murphy TH, Hayden MR, Raymond LA (2004) Enhanced striatal NR2B-containing N-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. J Neurophysiol 92:2738–2746. doi:10.1152/jn.00308.2004

Lin B, Rommens JM et al (1993) Differential 3′ polyadenylation of the Huntington disease gene results in two mRNA species with variable tissue expression. Hum Mol Genet 2 (10):1541–1545

Lin B, Nasir J, Kalchman MA et al (1995) Structural analysis of the 5′ region of mouse and human Huntington disease genes reveals conservation of putative promoter region and di- and trinucleotide polymorphisms. Genomics 25:707–715

Liot G, Zala D, Pla P et al (2013) Mutant Huntingtin alters retrograde transport of TrkB receptors in striatal dendrites. J Neurosci 33:6298–6309. doi:10.1523/JNEUROSCI.2033-12.2013

Luo S, Vacher C, Davies JE, Rubinsztein DC (2005) Cdk5 phosphorylation of huntingtin reduces its cleavage by caspases: implications for mutant huntingtin toxicity. J Cell Biol 169:647–656. doi:10.1083/jcb.200412071

MacDonald ME, Vonsattel JP, Shrinidhi J et al (1999) Evidence for the GluR6 gene associated with younger onset age of Huntington's disease. Neurology 53:1330–1332
Mahant N, McCusker EA, Byth K et al (2003) Huntington's disease: clinical correlates of disability and progression. Neurology 61:1085–1092
McGeer EG, McGeer PL (1976) Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature 263:517–519
Mende-Mueller LM, Toneff T, Hwang SR et al (2001) Tissue-specific proteolysis of Huntingtin (htt) in human brain: evidence of enhanced levels of N- and C-terminal htt fragments in Huntington's disease striatum. J Neurosci 21:1830–1837
Milakovic T, Quintanilla RA, Johnson GVW (2006) Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells: functional consequences. J Biol Chem 281:34785–34795. doi:10.1074/jbc.M603845200
Mizuno K, Carnahan J, Nawa H (1994) Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. Dev Biol 165:243–256. doi:10.1006/dbio.1994.1250
Morton AJ, Edwardson JM (2001) Progressive depletion of complexin II in a transgenic mouse model of Huntington's disease. J Neurochem 76:166–172
Myers RH, Leavitt J, Farrer LA et al (1989) Homozygote for Huntington disease. Am J Hum Genet 45:615–618
Mykowska A, Sobczak K, Wojciechowska M et al (2011) CAG repeats mimic CUG repeats in the misregulation of alternative splicing. Nucleic Acids Res 39:8938–8951. doi:10.1093/nar/gkr608
Nasir J, Floresco SB, O'Kusky JR et al (1995) Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell 81:811–823
Panov AV, Burke JR, Strittmatter WJ, Greenamyre JT (2003) In vitro effects of polyglutamine tracts on Ca2+-dependent depolarization of rat and human mitochondria: relevance to Huntington's disease. Arch Biochem Biophys 410:1–6
Pineda JR, Canals JM, Bosch M et al (2005) Brain-derived neurotrophic factor modulates dopaminergic deficits in a transgenic mouse model of Huntington's disease. J Neurochem 93:1057–1068. doi:10.1111/j.1471-4159.2005.03047.x
Pringsheim T, Wiltshire K, Day L et al (2012) The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. Mov Disord 27:1083–1091. doi:10.1002/mds.25075
Reiner A, Dragatsis I, Zeitlin S, Goldowitz D (2003) Wild-type huntingtin plays a role in brain development and neuronal survival. Mol Neurobiol 28:259–276. doi:10.1385/MN:28:3:259
Rigamonti D, Bauer JH, De-Fraja C et al (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. J Neurosci 20:3705–3713
Rigamonti D, Sipione S, Goffredo D et al (2001) Huntingtin's neuroprotective activity occurs via inhibition of procaspase-9 processing. J Biol Chem 276:14545–14548. doi:10.1074/jbc.C100044200
Sathasivam K, Neueder A, Gipson TA et al (2013) Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc Natl Acad Sci USA 110:2366–2370. doi:10.1073/pnas.1221891110
Schwarcz R, Foster AC, French ED et al (1984) Excitotoxic models for neurodegenerative disorders. Life Sci 35:19–32
Sheng M, Kim MJ (2002) Postsynaptic signaling and plasticity mechanisms. Science 298:776–780. doi:10.1126/science.1075333
Shimohata T, Nakajima T, Yamada M et al (2000) Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. Nat Genet 26:29–36. doi:10.1038/79139
Smith R, Petersén A, Bates GP et al (2005) Depletion of rabphilin 3A in a transgenic mouse model (R6/1) of Huntington's disease, a possible culprit in synaptic dysfunction. Neurobiol Dis 20:673–684. doi:10.1016/j.nbd.2005.05.008

Sobczak K, de Mezer M, Michlewski G et al (2003) RNA structure of trinucleotide repeats associated with human neurological diseases. Nucleic Acids Res 31:5469–5482

Spargo E, Everall IP, Lantos PL (1993) Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. J Neurol Neurosurg Psychiatr 56:487–491

Steffan JS, Kazantsev A, Spasic-Boskovic O et al (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci USA 97:6763–6768. doi:10.1073/pnas.100110097

Steffan JS, Agrawal N, Pallos J et al (2004) SUMO modification of Huntingtin and Huntington's disease pathology. Science 304:100–104. doi:10.1126/science.1092194

Takano H, Gusella JF (2002) The predominantly HEAT-like motif structure of huntingtin and its association and coincident nuclear entry with dorsal, an NF-kB/Rel/dorsal family transcription factor. BMC Neurosci 3:15

Tanaka K, Shouguchi-Miyata J, Miyamoto N, Ikeda J-E (2004) Novel nuclear shuttle proteins, HDBP1 and HDBP2, bind to neuronal cell-specific cis-regulatory element in the promoter for the human Huntington's disease gene. J Biol Chem 279:7275–7286. doi:10.1074/jbc.M310726200

Tartari M, Gissi C, Sardo Lo V et al (2008) Phylogenetic comparison of huntingtin homologues reveals the appearance of a primitive polyQ in sea urchin. Mol Biol Evol 25:330–338. doi:10.1093/molbev/msm258

The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72(6):971–983

Van Raamsdonk JM, Pearson J, Rogers DA et al (2005a) Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. Hum Mol Genet 14:1379–1392. doi:10.1093/hmg/ddi147

Van Raamsdonk JM, Pearson J, Slow EJ et al (2005b) Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci 25:4169–4180. doi:10.1523/JNEUROSCI.0590-05.2005

Van Raamsdonk JM, Murphy Z, Selva DM et al (2007) Testicular degeneration in Huntington disease. Neurobiol Dis 26:512–520. doi:10.1016/j.nbd.2007.01.006

Ventimiglia R, Mather PE, Jones BE, Lindsay RM (1995) The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 7:213–222

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384

Wang H, Lim PJ, Karbowski M, Monteiro MJ (2009) Effects of overexpression of huntingtin proteins on mitochondrial integrity. Hum Mol Genet 18:737–752. doi:10.1093/hmg/ddn404

Wang R, Luo Y, Ly PTT et al (2012) Sp1 regulates human huntingtin gene expression. J Mol Neurosci. doi:10.1007/s12031-012-9739-z

Warby SC, Chan EY, Metzler M et al (2005) Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. Hum Mol Genet 14:1569–1577. doi:10.1093/hmg/ddi165

Warby SC, Montpetit A, Hayden AR et al (2009) CAG expansion in the Huntington disease gene is associated with a specific and targetable predisposing haplogroup. Am J Hum Genet 84:351–366. doi:10.1016/j.ajhg.2009.02.003

Wellington CL, Ellerby LM, Hackam AS et al (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. J Biol Chem 273:9158–9167

Wexler NS, Young AB, Tanzi RE et al (1987) Homozygotes for Huntington's disease. Nature 326:194–197. doi:10.1038/326194a0

White JK, Auerbach W, Duyao MP et al (1997) Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17:404–410. doi:10.1038/ng1297-404

Xia J, Lee DH, Taylor J et al (2003) Huntingtin contains a highly conserved nuclear export signal. Hum Mol Genet 12:1393–1403

Young FB, Butland SL, Sanders SS et al (2012) Putting proteins in their place: palmitoylation in Huntington disease and other neuropsychiatric diseases. Prog Neurobiol 97:220–238. doi:10.1016/j.pneurobio.2011.11.002

Yu Z, Teng X, Bonini NM (2011) Triplet repeat-derived siRNAs enhance RNA-mediated toxicity in a Drosophila model for myotonic dystrophy. PLoS Genet 7:e1001340. doi:10.1371/journal.pgen.1001340

Zeitlin S, Liu JP, Chapman DL et al (1995) Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. Nat Genet 11:155–163. doi:10.1038/ng1095-155

Zhang Y, Li M, Drozda M et al (2003) Depletion of wild-type huntingtin in mouse models of neurologic diseases. J Neurochem 87:101–106

Zuccato C, Ciammola A, Rigamonti D et al (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293:493–498. doi:10.1126/science.1059581

Zuccato C, Tartari M, Crotti A et al (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35:76–83. doi:10.1038/ng1219

Zuccato C, Belyaev N, Conforti P et al (2007) Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. J Neurosci 27:6972–6983. doi:10.1523/JNEUROSCI.4278-06.2007

Mouse Models of Huntington's Disease

Simon P. Brooks and Stephen B. Dunnett

Abstract In this review, we explore the similarities and differences in the behavioural neurobiology found in the mouse models of Huntington's disease (HD) and the human disease state. The review is organised with a comparative focus on the functional domains of motor control, cognition and behavioural disturbance (akin to psychiatric disturbance in people) and how our knowledge of the underlying physiological changes that are manifest in the HD mouse lines correspond to those seen in the HD clinical population. The review is framed in terms of functional circuitry and neurotransmitter systems and how abnormalities in these systems impact on the behavioural readouts across the mouse lines and how these may correspond to the deficits observed in people. In addition, interpretational issues associated with the data from animal studies are discussed.

Keywords Behaviour · Mouse · Huntington's disease · Neuropathology · Motor · Cognitive · Psychiatric

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S. P. Brooks (✉) · S. B. Dunnett
Brain Repair Group, Division of Neuroscience, Cardiff University School of Bioscience, Museum Avenue, Cardiff, Wales, UK
e-mail: BrooksSP@cardiff.ac.uk

S. B. Dunnett
e-mail: DunnettSB@cardiff.ac.uk

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1 Introduction

Huntington's disease (HD) is caused by an inherited mutation in the gene that codes for the protein huntingtin (The Huntington's disease collaborative research group 1993), resulting in a mutant protein (mHtt) containing an expanded polyglutamine length. This CAG expansion induces a toxic gain of function when expanded over the critical disease penetrance threshold of 39 CAG repeats. This single mutation induces a distinctive neuropathological profile which, at a structural level, is characterised primarily by the early loss of medium spiny neurons (MSNs) from the caudate nucleus and progressive cortical atrophy (Vonsattel et al. 1985; de la Monte et al. 1988; Graveland et al. 1985; Myers et al. 1991; Rosas et al. 2005), along with white matter atrophy (Paulsen et al. 2006, 2010; Tabrizi et al. 2011). Moreover, at a cellular level, the aggregation of mutant protein into insoluble aggregates within striatal and cortical regions yields proteinaceous inclusions in affected neurons (Gomez-Tortosa et al. 2001; DiFiglia et al. 1997; Roizin et al. 1974). As the disease progresses, the cellular neurodegeneration spreads to other structures within and beyond the basal ganglia, such as the putamen and cerebellum (Aylward et al. 2011; Braak and Braak 1992a; Gomez-Tortosa et al. 2001; Rosas et al. 2002; Heinsen et al. 1999; Rub et al. 2013). As the focal point of the disease is the caudate nucleus, loss of these cells is associated with a behavioural syndrome characterised by deficits in corticostriatal-mediated functions within the motor, cognitive and psychiatric domains which become manifest from the earliest stage of the disease (Lawrence et al. 1996, 1998, 1999, 2000; Snowden et al. 1998, 2001, 2002, 2003, 2008; Stout and Johnson 2005; Thompson et al. 2002, 2010, 2012; Labuschagne et al. 2013; Novak et al. 2012; Stout et al. 2012; Tabrizi et al. 2013). Disease progression broadens the spectrum of functional deficits as more brain regions become affected. Death usually occurs between 15 and 20 years from clinical diagnosis (Warby et al. 2008).

As the disease is caused by a single mutation it is highly amenable to modelling in rodents, resulting in a number of mouse lines that were created with a variety of constructs and promoters (see Table 1), around 10 of which are in widespread use (Gray et al. 2008; Lin et al. 2001; Mangiarini et al. 1996; Menalled et al. 2003, 2012; Schilling et al. 2007; Slow et al. 2003; Wheeler et al. 1999). Many of these mouse lines demonstrate significant similarity at the behavioural, anatomical and gene expression level with the human condition, but also significant differences. One of the key issues in the field is to determine the neurobiological substrates that underlie the behavioural observations that are reported. The purpose of this chapter is to place mouse models of the disease into the context of the human condition; we will focus on the motor and cognitive deficits and underlying aberrant neuronal circuitry found in the HD mouse lines, and how these deficits relate to what we know of the neurobiology of the disease in humans. We start the review with a brief consideration of issues associated with the interpretation of mouse behavioural data, followed by an outline of the neuropathology of the HD basal ganglia, which then provides the context for the succeeding sections on mouse behavioural neurobiology.

Table 1 The most commonly used HD mouse lines

Model	Initial reference	[a]CAG length	Insert	Promoter	Symptom manifestation (example ref)			Onset of NIIs	[b]Spread of NIIs	Striatal atrophy
					Motor	Cogn.	Behav.			
R6/1	128	115	Fragment (67 aa N-terminus)	*HTT*	Early (30)	Early (30)	Early (173)	<6 weeks	Widespread	16 weeks
R6/2	128	145	Fragment (67 aa N-terminus)	*HTT*	Early (38)	Early (125)	Early (243)	<6 weeks	Widespread	13 weeks
HdhQ92	237	90	Full length (chimeric exon 1)	*Htt*	Late (218)	Early (216)	Late (218)	40 weeks	Striatal	Absent
HdhQ111	237	109	Full length (chimeric exon 1)	*Htt*	Late (238)	No report	Early (152)	24 weeks	Widespread	Absent
HdhQ150	123	150	Complete (chimeric exon 1)	*Htt*	Mid (123)	Mid (26)	No report	20 weeks	Widespread	100 weeks
HdhQ140	137	140	Full length (chimeric exon 1)	*Htt*	Early (137)	Early (95)	Early (95)	16 weeks	Widespread	110 weeks
YAC128	192	128	Full length (human)	*HTT and regulatory elements*	Early (43)	Early (43)	Early (43)	56 weeks	Widespread	12 weeks
N171-82Q	188	82	Fragment (171 aa N-terminus)	*Prp*	Early (43)	Early (43)	Early (43)	24 weeks	Widespread	10 weeks
BACHD	84	97	Full length (human)	*HTT and regulatory elements*	Early (84)	Early (1)	Early (98)	56 weeks	Striatal Cortical	56 weeks
zQ175	136	188	Full length (chimeric exon 1)	*Htt*	Early (136)	Mid (91)	*No report*	N/D	N/D	12 weeks

[a] denotes ~CAG lengths in original studies that may drift with time

[b] denotes spread of inclusions based on reports that may not include assessments beyond the striatum and cortex, and "Widespread" denotes spread of NIIs beyond the striatum and cortex (*Cogn.* cognitive, *Behav.* behavioural, *HTT* human huntingtin promotor, *Htt* mouse huntingtin promotor, *Prp* mouse prion promotor, *N/D* no available data)

2 Interpreting and Understanding Mouse Behavioural Readouts

The neurobiological interpretation of motor dysfunction is confounded by the broad nature of the motor system. For example, if we consider fine motor control, we consider signals from the motor cortex being transmitted to the striatum and thalamus where they are then sent to the cerebellum and/or descending motor neurons, and then out to muscle. Cellular abnormalities at any point in this pathway can disrupt motor performance, and whilst neurological examination in humans may provide clues to the underlying locus of neuropathology, this is generally not possible in rodents. As a result, the readouts from motor tasks in rodents are always open to a number of interpretations, with researchers generally choosing the explanation that is most in-keeping with the disease under investigation, often regardless of evidence of broader pathology in the model. In contrast, the neurobiology of cognitive deficits is more easily interpreted if the study is well designed, taking account of motor disabilities for example. The circuits and brain structures that underlie cognitive function tend to be well-defined through lesion and pharmacological studies that may demonstrate specific patterns of performance deficit if disrupted by disease.

In HD, one of the current focusses of behavioural research is on the psychiatric aspects of the disease. In this domain, core features of HD have been identified with apathy and depression being the most predominant, but with others such as irritability being common. In mouse models, these "behavioural" abnormalities are difficult to probe due to the human nature of the target phenomena, but tests of motivation and learned helplessness (behavioural despair) have been used in a number of mouse lines, and it is clear from the literature that the underlying neurochemical substrates (dopamine, glutamate, etc.) that mediate many of these deficits are compromised in HD, as they are also in the mouse models (see below). Unfortunately, interpretation of the results in the handful of relevant reports with mice is difficult due to contamination by motor dysfunction in the most commonly applied test of "depression-like" behaviour (the Porsolt forced swim task). The absence of studies using more specific tests relevant to human affective or behavioural deficit in mice means that the final section of this review is brief.

Finally, it should be noted that in reality the distinction among motor, cognitive and psychiatric (behavioural) abnormalities is somewhat artificial, providing a framework that allows us to conceptualise the different components of the disease, but involving functional domains that overlap significantly. Dysfunction in a single neuronal pathway is likely to contribute to the deficits across all functional domains with functional readouts never being purely motoric, cognitive or behavioural in nature. This said, with ongoing test development, the functional readouts are becoming ever more refined and the work currently being produced in HD mouse lines is often validated with secondary tests or pharmacological probes to provide behavioural data with good face and predictive validity. A brief overview of the human condition is presented below to provide a comparable context for following the mouse studies.

3 The Neurobiology of Movement in Huntington's Disease

In primates the basal ganglia comprise of the neostriatum (caudate nucleus and putamen divided by the internal capsule), the ventral striatum (nucleus accumbens and olfactory tubercle), the internal and external globus pallidus and the ventral pallidum, the substantia nigra and the subthalamic nucleus. The pathways innervating these structures are highly conserved between mammalian species (see Fig. 1a for the rodent brain). A first-order overview of the striatum would be to view it as the major relay for descending cortical afferent neurons whose information is channelled back to the cortex or to more distal brain regions via the thalamus. However, within this simplified framework, there are pathways organised as distinct parallel circuits subserving operationally distinct behavioural facets, mediated by the integration of cortical information that is then segregated to form distinct descending cortico-striatal loops (Draganski et al. 2008; Lehericy et al. 2004; Alexander et al. 1986, 1990). These pathways are then channelled via the inhibitory GABAergic medium spiny output neurons of the basal ganglia to the thalamus via the striatal "direct" pathway:

$$\text{caudate}\,[\textit{inhibitory}] \rightarrow \text{globus pallidus } \textit{parsinterna}\,[\textit{inhibitory}] \rightarrow \text{thalamus}\,[\textit{excitatory}]$$

and "indirect" pathway:

$$\text{caudate}\,[\textit{inhibitory}] \rightarrow \text{globus pallidus } \textit{parsexterna}\,[\textit{inhibitory}] \rightarrow \text{subthalamicnucleus}\,[\textit{excitatory}] \rightarrow \text{globuspallidus } \textit{parsinterna}\,[\textit{inhibitory}] \rightarrow \text{thalamus}\,[\textit{excitatory}]$$

where they synapse at specific thalamic nuclei, thus defining the segregation of the distinct cortico-striatal loops. This information is fed back to the cortex via ascending excitatory axons that form feedback loops that regulate the cortical source of each loop as well as open loop connections to output areas of premotor and motor cortex and other brain areas such as the cerebellum and brainstem.

In HD brains, the reduction in dopamine D2 receptors has been considered as an early marker of MSN dysregulation (Crook and Housman 2012). The selective degeneration of enkephalin rich and dopamine D2 receptor containing neurons from the caudate nucleus to the globus pallidus external reduces the inhibitory control within the indirect pathway, such that the subthalamic nucleus becomes more inhibited, reducing excitatory stimulation to the globus pallidus internal which in turn has less inhibitory influence on the thalamus. This results in increased glutamatergic feedback to the cortex which itself becomes overactive, setting in place an overactive cortico-striato-thalamic feedback loop, resulting in choreic movements. As the disease progresses, the choreic movements often disappear to be replaced with hypoactivity, reflecting a more widespread and less specific striatal neurodegeneration that includes the loss of MSNs in the dopamine D1 rich direct pathway, which induces an overarching loss of motor tone throughout the motor circuits.

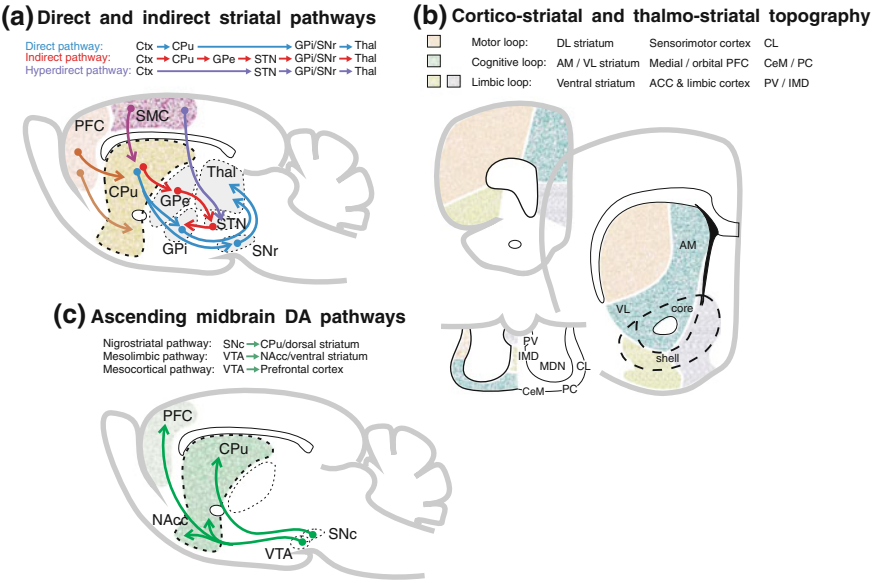

Fig. 1 Simplified schematics of the neuroanatomical pathways of the rodent cortex and basal ganglia. **a** The direct, indirect and hyperdirect pathways of the basal ganglia depicting the organisation of corticostraital innnervation of thalamic nuclei, via specific striatal regions. **b** Generalised topographical map of the functional relationship between the frontal cortex and the striatum, illustrating motor, cognitive and behavioural (limbic) connectivity to thalamic nuclei. **c** The ascending DA pathways that innervate the striatum and prefrontal cortex. *ACC* nucleus accumbens, *AM* amygdala, *CL* central lateral thalamic nucleus, *Ctx* cortex, *CPu* caudate-putamen, *DL* dorsolateral, *GPe* globus pallidus external, *GPi* globus pallidus internal, *IMD* interomediodorsal thalamic nucleus, *MDN* medial dorsal nucleus, *NAcc* nucleus accumbens, *PFC* prefrontal cortex, *PV* paraventricular thalamic nucleus, *SNc* substantia nigra pars compacta, *SNr* substantia nigra, *STN* subthalamic nuclei, *Thal* thalamus, *VL* ventrolateral striatum, *VTA* ventral tegmental area. (For reviews see Alexander et al. 1986, 1990; Bjorklund and Dunnett 2007; Voorn et al. 2004)

Whilst the striatal neurodegeneration may be the initial site and most severely affected brain region for HD neuropathology, the cerebral cortex is also recognised as being severely affected at the early disease stage (Braak and Braak 1992a, b; Heinsen et al. 1994; Rosas et al. 2002, 2005; Paulsen et al. 2006). Cortical cell loss is most pronounced in the supragranuler layers of Brodmann's areas, 1–3, 17 and 41 with cell loss most pronounced in layer III but also layer V (Heinsen et al. 1994). Precisely how this affects the development of motor dysfunction at the different stages of disease [Vonsattel stages (Vonsattel et al. 1985)] is not known, but it is likely that overt cell loss contributes to the hypoactivity of the later disease stages through degradation of the descending glutamatergic tone. In addition to the degeneration and/or atrophy of cortical and basal ganglia regions, there are reports of thalamic and cerebellar neuron loss (Heinsen et al. 1999; Schmitz et al. 1999; Rub et al. 2013), with cerebellar atrophy not correlating with the extent of striatal

atrophy, but present in grade 2 brains and demonstrating neuronal intra-nuclear inclusions (NIIs) (Rub et al. 2013).

Post-mortem studies suggest that the ascending nigrostriatal dopamine system is intact in HD patients (Bird et al. 1980; Spokes 1980; Mann 1989), and that dopamine-related movement problems in early disease stages are not related to nigral degeneration per se but rather to loss of post-synaptic striatal neurons expressing high levels of both D1 and D2 dopamine receptors (Kurlan et al. 1988). However, reductions in dopamine D1 receptors on substantia nigra neurons have been reported (Filloux et al. 1990; Cross and Rossor 1983), as has the wholesale loss of CB1 receptors from the substantia nigra pars reticulate (Glass et al. 1993). Whether the reductions in D1 and CB1 receptors in turn influence the progression of neuropathology is not known.

In rodents, the internal capsule fibres that subdivide regions of the striatum into discrete caudate and putamen nuclei in the human is arranged not as a sheet but as multiple pencil-like fibre bundles, resulting in a single nucleus, the 'striatum'. The rodent striatum incorporates the caudate and putamen (dorsal, 'caudate-putamen' or 'neostriatum'), and the nucleus accumbens and olfactory tubercle (or 'ventral striatum'), which has comparable functionality to the human striatal complex, validating the use of rodent models for modelling basal ganglia disorders. Conversely, the closely associated internal and external parts of the globus pallidus that make up a single structural nucleus in primates and carnivores, is more discretely subdivided between the globus pallidus and entopeduncular nucleus in rodents (equivalent to the *pars externa* and *pars interna*, respectively, of humans). Nevertheless, it should be remembered that in spite of gross differences in macroscopic anatomy, rodents and humans show similar organisation of the basal ganglia at the levels of cell type, the pathways of axonal connectivity and molecules of neurochemical signalling, providing conservation of functional pathways (Fig. 1b).

4 The Neurobiology of Motor Dysfunction in Mouse Models of HD

Many mouse models of HD share many of the characteristics of the disease—including mHtt aggregation, inclusion formations, cortical/striatal atrophy and overt neuronal cell loss (Bayram-Weston et al. 2012a, b, c, d; Davies et al. 1997; Heikkinen et al. 2012; Hickey et al. 2008; Mangiarini et al. 1996; Morton et al. 2000; Trueman et al. 2008; Van Raamsdonk et al. 2005a, b; Wheeler et al. 2000, 2002). Most of these mouse lines demonstrate pronounced behavioural disturbance prior to reported cell loss (Brooks et al. 2012c; Lin et al. 2001; Mangiarini et al. 1996; Trueman et al. 2008, 2012b). Similarly, there is considerable debate relating to the role of the aggregation and inclusion formations in the mouse brain, with the currently predominant conceptualisation being that NIIs are manifestations of cell survival mechanisms with the real pathological mechanism of the cell being more

closely aligned with the soluble species of mHtt during the initial aggregation stage (Miller et al. 2010; Saudou et al. 1998). As with cell loss, it is clear that behavioural dysfunction can occur in advance of the signs of overt aggregation neuropathology (Trueman et al. 2012b; Reddy et al. 1999), leading to the notion that early stage disease is characterised by synaptic pathology—whether in the pre-synaptic terminal or post-synaptic receptor changes (Gibson et al. 2005; Twelvetrees et al. 2010; Ariano et al. 2002; Benn et al. 2005; Nithianantharajah et al. 2008)—as well as other cellular process not directly related to the aggregation process per se. An important study using the "shortstop" mouse demonstrated widespread inclusion formation in the absence of behavioural symptoms (Slow et al. 2005). In fact, studies on the human HD brain demonstrate relatively few inclusions compared to the murine models (DiFiglia et al. 1997; Roizin et al. 1974; Bayram-Weston et al. 2012a, b, d). At the very least, aggregation of mHtt in the mouse brain permits us to determine which populations of cells are affected at any given time and allow us to map the spread of neuropathology throughout the mouse brain, which can help to target behavioural assessments that probe for specific abnormalities in affected circuits. With this in mind, the following section examines the neurobiology of HD mouse lines in terms of dysfunction in neuronal circuitry (Fig. 1a–c) with only the occasional reference to the effects of gross cell loss or inclusion pathology.

Whilst there is clearly significant anatomical conservation between mammalian species, there are substantial differences between the manifestation of HD in people and the equivalent genetic syndrome as modelled in rodents. The most obvious difference is that rodent models, whether lesion-based or genetically modified, do not develop the choreic movements observed in people, although there are reports of chorea in the highly inbred tgHD rat model (Temel et al. 2006). However, there are many similarities between the rodent models and the human condition, including the behavioural findings from HD mouse lines and people that increased motor activation (chorea in patients) gives way to hypoactivity (apathy in patients) as the disease advances (Menalled et al. 2003; Slow et al. 2003; Reddy et al. 1999; Thompson et al. 2012). Evidence from an electrophysiological study using corticostriatal projection neurons derived from YAC128 mice demonstrated increased synaptic currents and glutamate release from 1 month of age which later becomes reduced as the mice age (Joshi et al. 2009), consistent with the notion that changes in striatal outputs drive motor signals though the cortex which is expressed as disease-mediated changes in motor activation. In the case of the YAC128 and HD89 mice, hyperactivity at a young age was reported that was superceded by hypoactivity as the disease progressed (Slow et al. 2003; Reddy et al. 1999), as was also reported for the HdhQ140 mice (Menalled et al. 2003). However, such results tend to be inconsistently reported (Menalled et al. 2009; Rising et al. 2011), with most HD mouse lines demonstrating a progressive decline in motor activity levels as the disease progresses (Menalled et al. 2009; Heikkinen et al. 2012; Rising et al. 2011). Consistent with the notion of disinhibition of striatal activity underpinning hyperactivity, one study found increased spontaneous GABAergic excitability in striatal tissue in both the knock-in zQ175 and the R6/2

mouse lines (Dvorzhak et al. 2013), with increased excitability in the striatal output neurons in zQ175 also being reported elsewhere (Heikkinen et al. 2012). It should be noted that both the R6/2 and zQ175 lines fail to demonstrate the initial motor hyperactivity demonstrated in the YAC128 and HdhQ140 lines. Nevertheless, increased glutamatergic neurotransmission through the cortex should correspond to increased activity levels and vice versa, and since HD mouse lines typically demonstrate reduced motor activation especially at late stages in the disease, it should be expected that this would correspond to reduced cortical input and output to the striatum. Several mouse lines demonstrate such an effect.

BACHD mice demonstrate a progressive decline in cortical activity from 3 months of age including reduced excitation prior to neuronal degeneration, which corresponded to the onset of motor dysfunction (Spampanato et al. 2008). Cortico-striatal dysfunction was found to be both AMPA and NMDA receptor mediated in YAC128 mice (Milnerwood and Raymond 2007), as also reported for the R6/2 mouse where reductions in glutamate receptor (AMPA and NMDA) sensitivity were also present in cortical pyramidal cells at an early age (Andre et al. 2006). This dysfunction may be directly related to the mutant protein aggregation in the cortical cells, rather than a downstream effect of striatal pathology since expressing mutant Htt in neuronal cultures has been demonstrated to decrease the frequency and amplitude of AMPA receptor-mediated synaptic transmission (Mandal et al. 2011), and in the R6 mouse lines inclusion pathology is present throughout the brain, including the cortex from an early age (Bayram-Weston et al. 2012b; Davies et al. 1997; Mangiarini et al. 1996). At the level of the striatum, symptomatic R6/2 mice had marked passive and active MSN membrane potential abnormalities including abnormal action potentials (Klapstein et al. 2001), and both knock-in (CAG71 and CAG94) and transgenic (R6/2, YAC128) mouse models have been found to be more sensitive to striatal NMDA receptor activation than their wild-type littermates, suggesting that there are changes in NMDA receptor function within striatal tissue (Cepeda et al. 2001; Levine et al. 1999; Starling et al. 2005), in contrast to the decreased sensitivity in glutamate-mediated neurotransmission found in the N171-Q82 neurons (Mandal et al. 2011). One study was able to dissect NMDA-mediated toxicity from AMPA-mediated toxicity and found that R6/1 and R6/2 mice were resistant to striatal NMDA but not AMPA stimulation at the point where nuclear inclusions and behavioural deficits became present (Hansson et al. 2001). Whilst the different mouse lines demonstrate abnormalities in striatal and corticostriatal functioning, there is also great consistency suggesting glutamate-mediated mechanisms are central to this dysfunction, and one study running a direct comparison between a transgenic (YAC128) line and a knock-in (HdhQ140) mouse line found considerable similarities in MSN dysfunction which included a lower frequency of spontaneous excitatory postsynaptic currents and a greater frequency of inhibitory postsynaptic currents (Cummings et al. 2010). It should be considered that general levels of activity through the striatal output pathways are not simply related to glutamatergic mechanisms alone but may also involve abnormalities within the MSNs that may have non-glutamatergic causes such as disrupted trafficking of GABA receptors,

as has been found in the N171-Q82 mouse line (Yuen et al. 2012). In addition, other key modulators of striatal activity such as the dopamine and cholinergic systems have also been found to be disrupted in HD mouse models with modulators of these systems being used as therapeutic agents such as tetrabenazine (de Tommaso et al. 2011; Pidgeon and Rickards 2013).

The selective loss of ascending nigrostriatal dopaminergic input into the striatum induces the poverty of movement seen in Parkinson's disease and may contribute to motor output in HD. Theoretically MSN cell loss could result in loss of dopamine sensitivity and alterations in dopamine-mediated GABAergic neurotransmission in the absence of abnormalities in ascending nigrostriatal neurons, but extracellular striatal dopamine levels have been found to be decreased in R6/2 and YAC128 mice as was dopamine release in response to an amphetamine challenge (Callahan and Abercrombie 2011), suggesting that in some mouse lines this is not the case. Similarly, dopamine levels were found to be reduced in R6/2 mice from 8 weeks of age with the dopamine metabolites 3-methoxytyramine and homovanillic acid reduced in R6/2 mice at 4 weeks of age, when they are still motorically asymptomatic (Mochel et al. 2011), consistent with other studies in this strain (Ortiz et al. 2010; Hickey et al. 2002). Reductions in dopamine activity were linked in some studies to a decline in motor performance in the R6/2 mice including levels of motor activity, grooming and rotarod performance (Callahan and Abercrombie 2011; Hickey et al. 2002). One study in BACHD mice has demonstrated that early disease stereotypy was mediated by D1 receptor activation, which could be corrected with the administration of tetrabenazine or a dopamine D1 antagonist (Andre et al. 2011), Moreover, R6/2 mice have been found to have reduced LTP in dopamine sensitive cells (Kung et al. 2007). However, it should be noted that a study examining striatal dopamine uptake by microdialysis in freely moving R6/2 mice, found no difference between wild type and R6/2 carriers. In this study, greater inhibition of the evoked release of acetylcholine rather than dopamine was found in striatal tissue, suggesting cholinergic interneurons were more sensitive to the disease (Vetter et al. 2003). It may be that dopamine stimulation differentially effects cholinergic activity in wild type and R6/2 mice since striatal cholinergic efflux was found to be unchanged in response to a GABA agonist, but was reduced in R6/2 mice after a systemic injection of a dopamine D1 agonist (Farrar et al. 2011). So, whilst it appears that the mutant Htt affects the integrity of descending glutamatergic neurotransmission in corticostriatal neurons, dysfunction in ascending nigrostriatal efferent fibres and changes in the sensitivity of striatal dopamine receptors are also key components of the aberrant motor system in HD mouse models.

To further complicate this picture, loss of cannabinoid CB1 receptors, which are highly expressed throughout the intact striatum, are one of the earliest postmortem markers of HD pathology (Allen et al. 2009; Glass et al. 2000; Van Laere et al. 2010), a finding that has been replicated in the R6/1 (Dowie et al. 2009; Glass et al. 2004), R6/2 (Bari et al. 2013; Chiodi et al. 2012) and YAC128 mouse lines, but not in the BACHD mouse (Pouladi et al. 2012). Knockout of CB1 receptors has been shown to worsen the motor performance on the rotarod in the N171-82Q

mouse line (Mievis et al. 2011). However, despite the clear evidence that CB1 receptors are lost in HD and mouse models of the disease, treatment of R6/1 mice with cannabinoid agonists has not been found to be an effective strategy to modify the deleterious progression of motor symptoms (Dowie et al. 2010), suggesting that the specific activation of CB1 receptors may not greatly influence motor aspects of the disease.

In patients and HD mouse models alike, a reduction in striatal brain-derived neurotrophic factor (BDNF) has also been reported (Zuccato et al. 2001, 2005, 2008; Canals et al. 2004; Giralt et al. 2009, 2011a; Griffioen et al. 2012; Jiang et al. 2013; Reiner et al. 2012b; Samadi et al. 2013; Xie et al. 2010). Various lines of evidence suggest that loss of this growth factor is implicated in the loss of striatal integrity through a number of different mechanisms including the lack of normal trophic support for striatal cells, and the transport and imbalance in the striatum of the BNDF receptor target, TrkB (Canals et al. 2004; Liot et al. 2013). Several mouse models have reported a reduction in BDNF in cortical or striatal tissue relative to their wild-type littermates: the R6/1 (Giralt et al. 2009; Spires et al. 2004; Canals et al. 2004) and R6/2 (Giralt et al. 2011a; Reiner et al. 2012b; Samadi et al. 2013) lines, the YAC128 line (Xie et al. 2010), the N171-82Q line (Griffioen et al. 2012; Jiang et al. 2013) and the BACHD mouse (Gray et al. 2008), making them useful lines for therapeutic trials directed at BDNF replacement or stimulation of the TrkB receptor (Jiang et al. 2013; Xie et al. 2010; Giralt et al. 2009, 2011a; Reiner et al. 2012b). Loss of BDNF in the R6/2 and R6/1 brain has been found to be closely correlated to the manifestation and severity of motor deficits (Samadi et al. 2013; Canals et al. 2004). Unlike glutamate, GABA, acetylcholine and dopamine, BDNF is probably not directly involved in the modulation of the motor system, but offers neuroprotection through trophic support. It is of interest that a conditional cortical knockout of BDNF induced a clasping phenotype and striatal atrophy with MSN cell loss (Baquet et al. 2004), consistent with the clasping phenotype observed in several HD mouse lines including the HdhQ150 (Lin et al. 2001), R6/2 (Mangiarini et al. 1996; Deckel et al. 2002), R6/1 (Van Dellen et al. 2008), YAC128 (Dey et al. 2010) and CAG89 mice (Reddy et al. 1999).

Aside from the measure of gross motor activity, other signs of motor dysfunction are also present in the mouse lines that are less readily interpreted in terms of striatal function. The most commonly used behavioural probe in the HD field is the rotarod, which is generally believed to measure motor coordination as the mouse must adjust its stride pattern to remain on a rotating rod, which in most studies is accelerating. Rotarod deficits have been found in practically all HD mouse lines (Gray et al. 2008; Brooks et al. 2012a, b, e; Menalled et al. 2009; Slow et al. 2003; Pouladi et al. 2012; Carter et al. 1999; Hickey et al. 2008; Lin et al. 2001), with the observed deficits generally being attributed to striatal dysfunction. However, although the readout from this test is very clear there are many underlying aspects of pathology that could cause the mice to fail at the test, several of which that may not relate to striatal function. For example, inclusion formation in the cerebellum is common in most of the mouse lines, often at a young age, but is widely overlooked

when focussing on striatal loci for functional abnormalities to validate alternative HD strains as good disease models.

Some mouse lines also demonstrate peripheral pathology, such as atrophy and inclusion formations in muscle and other peripheral tissues (Moffitt et al. 2009; Ribchester et al. 2004). Nerve abnormalities have been found in the R6/2 mouse (Wade et al. 2008). The N171-82Q and R6/1 mice have been found to have heart rate abnormalities (Griffioen et al. 2012; Kiriazis et al. 2012), and abnormal glucose regulation (Martin et al. 2009; Josefsen et al. 2008), the latter problem being well described previously in the R6/2 mouse (Bjorkqvist et al. 2005; Hurlbert et al. 1999). Dysfunction in any or all of these peripheral systems could contribute to motor dysfunction. To further emphasise this point, the HdhQ92 mouse line has a highly specific pattern of aggregation and inclusion development that begins in the olfactory tubercle, piriform cortex and ventral striatum before 8 months of age, but which, even in aged mice, does not progress beyond the striatum; this mouse has no motor deficits until it is over 2 years of age (Brooks et al. 2012c), despite marked cognitive deficits on tasks that probe striatal function (Trueman et al. 2012a, b). This suggests that diffuse staining of mutant huntingtin in the striatum and the subsequent inclusion formations have no effect on motor readouts. Further, striatal atrophy and cell loss are frequently reported to be late onset events in some mouse lines and often do not correlate with the appearance of motor abnormalities. The results from all behavioural tests are open to a number of interpretations, but the more specific the aspect of behaviour being measured the clearer the interpretation of the results often is.

In this sense, aspects of the balance beam test which measures motor coordination and balance of the mouse as it crosses a narrow-raised bridge can be highly informative. Whilst the speed that the mouse crosses the bridge has been used as a measure of motor decline and balance in several HD lines including the R6 lines, YAC128, HdhQ150 lines (Brooks et al. 2012a, e; Carter et al. 1999; Heng et al. 2007), the number of foot-slips as a measure of fine motor control, has been found to offer a more sensitive and informative measure of performance in YAC128 and R6/1 mice (Brooks et al. 2012a, e). In humans, fine motor control is mediated at the level of the cortex, and the cerebellum regulates balance via thalamic and cortical outputs. A further insight into fine motor control can be gained through the use of runway tests with footprint analyses, which measure deterioration in gait as mice age (and notwithstanding other factors that may also influence performance, such as muscle tone and joint integrity). These tests have been found to be highly sensitive in several HD mouse lines including the R6/2 (Menalled et al. 2009; Carter et al. 1999; Pallier et al. 2009; Reiner et al. 2012a), HdhQ150 (Heng et al. 2007), HdhQ140 (Hickey et al. 2012) and the BACHD mouse (Abada et al. 2013).

Tests of the acoustic startle response and the effects of pre-pulse inhibition (PPI) provide another measure of reflexive motor activity that has proven sensitive in a broad range of HD mouse lines including the R6/1 and R6/2 lines, the HdhQ150, YAC128, BACHD and the N171-82Q line (Brooks et al. 2012a, b, e; Carter et al. 1999; Pouladi et al. 2012; Van Raamsdonk et al. 2005b; Norflus et al. 2004; Menalled et al. 2009). Although the interpretation of PPI deficits in terms of

a functional impairment in 'sensorimotor gating' can be difficult to dissect, the primary startle stimuli is an excellent measure of an automatic unconditioned sensorimotor reflex, and the pre-pulse aspect of the task provides a sensitive measure of the integration of motor systems with more sensory inputs into specific brain regions, including the striatum. Empirically, deficits on the startle aspect of the task denote abnormalities in the lower motor neurons and/or brain stem, whereas selective deficits in the prepulse trials as found in HD mice, do appear more specifically to reflect striatal dysfunction.

Other motor readouts tend to demonstrate more variation between studies largely due to the use of different tests used to measure the same phenomena, such as in grip strength tasks where grip strength metres, elevated wires, inverted screens or cage lids are used (Menalled et al. 2009; Brooks et al. 2012a, c; Hickey et al. 2012). To summarise this far, the use of motor tasks to determine changes in the neuropathology of HD mouse lines is informative but only in a very general way as they fail to probe the underlying neuroanatomical substrates with sufficient specificity, and arguably the real value of many of these tests is to provide a general disease-related readout for therapeutic trials. In contrast, the neurobiology of cognitive function has been very well defined in lesion and pharmacological studies making the interpretation of cognitive deficits in well-controlled and designed experiments more insightful when considering the underlying neurobiology.

5 The Neurobiology of Cognitive Dysfunction in Mouse Models of HD

With improved diagnostics and more sensitive clinical assessments, the chorea associated with HD is now considered to be a mid-stage disease characteristic. Increasingly, the focus of research in both the clinic and pre-clinical laboratory is the identification and characterisation of cognitive and psychiatric (behavioural) abnormalities, and it is certainly these functions that yield the greater disability and distress for patients and their families. Within the cognitive domain, short-term memory loss and attentional dysfunction are amongst the most widely reported deficits and appear relatively early in the disease development (Snowden et al. 2001; Sprengelmeyer et al. 1995; Thompson et al. 2012; Verny et al. 2007; Wolf et al. 2012), along with more disease-specific abnormalities such as set-shifting (Lawrence et al. 1996, 1998). The abnormalities in early stage disease are mediated particularly by the fronto-striato-thalamic circuits (Fig. 1b), and are consistent with theories and anatomical studies of disease progression (Alexander et al. 1986, 1990; Rosas et al. 2002, 2005; Tabrizi et al. 2011, 2013; Novak et al. 2012), and functional neuroanatomical studies designed to characterise (cortico) striatal function (Bussey et al. 1997; Clarke et al. 2008; Divac et al. 1967, 1978; Kim and Ragozzino 2005; Muir et al. 1993; Ragozzino et al. 2002; Rosvold and DELGADO 1956; Rosvold et al. 1958; White and Dunnett 2006). To aid conceptualisation of the role

of the dorsal regions of the striatum in cognitive function, it may be useful to consider the role as one of mediating choice selection and integrating cognitive function with motor output. Again, this is a simplification to aid our conceptualisation of the underlying processes and it should be considered that there are no real divisions among the motor, cognitive and psychiatric faculties as all are integrated to produce the functional outputs that are measured.

In mouse models of HD, the most consistently reported cognitive deficit is in reversal learning (Brooks et al. 2012d; Lione et al. 1999; Murphy et al. 2000; Abada et al. 2013; Van Raamsdonk et al. 2005b). In part, these findings probably reflect the ease with which the discrimination tests can be applied as they provide a simple means by which to produce datasets that probe specific aspects of the HD neuropathology. The circuitry underlying this deficit is well characterised with a particular role for the anterior cingulate and orbitofrontal cortices (Clarke et al. 2008; Furr et al. 2012; Kim and Ragozzino 2005; Klanker et al. 2013), which may mediate positive response feedback (Hampshire et al. 2012; Clarke et al. 2008), in the fronto-striatal-thalamic feedback loop. At the striatal level, lesions of the dorsomedial or medial striatum disrupt reversal learning (Clarke et al. 2008; Divac et al. 1978; McCool et al. 2008; Pisa and Cyr 1990; Ragozzino et al. 2002). As HD mice demonstrate neuropathology in the form of striatal and/or cortical atrophy or cell loss, or diffuse staining and/or inclusion formations in striatum and/or cortex (Bayram-Weston et al. a, b, c, d; Davies et al. 1997; Heikkinen et al. 2012; Hickey et al. 2008; Lin et al. 2001; Menalled et al. 2003; Reddy et al. 1999; Van Raamsdonk et al. 2005a, b; Lerner et al. 2012), the reversal learning task represents a task with good face validity for corticostriatal dysfunction. Reversal learning deficits are considered to be more of a mid-stage deficit in HD patients (Lawrence et al. 1996), whereas set-shifting deficits were considered to be early stage (Lawrence et al. 1998). In contrast to the reversal learning, set- and task-shifting deficits are mediated by similar neuronal substrates, mediated by more medial aspects of the prefrontal cortex (Bissonette et al. 2008; Floresco et al. 2008) that can loosely be described as measuring attentionally mediated cognitive flexibility. Both the HdhQ150 and the YAC128 lines have been found to have difficulty with the cognitive shifting types of task (Brooks et al. 2006, 2012d; Van Raamsdonk et al. 2005b).

Related to attentional shifts, another common component of the HD profile of cognitive deficits is attentional dysfunction (Josiassen et al. 1983; Lawrence et al. 1996; Roman et al. 1998; Sprengelmeyer et al. 1995). To study attentional dysfunction in mice requires specialist equipment and expertise, consequently there are relatively few studies in the HD mouse lines. A series of studies in the HdhQ92 mouse using an operant test in the 9-hole box chamber which was designed to probe for implicit learning deficits (Serial Implicit Learning Task—SILT), and based in part on the classic visuo-spatial attentional probe the 5-choice serial reaction time task (5-CSRTT), revealed attentional deficits from 4 months of age (Trueman et al. 2007, 2008, 2012a). Similar results were reported in the YAC128 mouse line (Brooks et al. 2012f). Different aspects of attentional function are mediated by different regions with the prefrontal cortex and basal ganglia (Chase et al. 2012; Lindgren et al. 2013; Muir et al. 1993; Agnoli et al. 2013) and involve a broad range

of neurotransmitter systems (Chudasama and Robbins 2006). One interesting paper that examined the role of attention in bimanual performance suggested that the cognitive deficits observed in patients may reflect the inability of patients to automate behaviours resulting in greater attentional loads required to perform normal tasks (Thompson et al. 2010), suggesting that attentional deficits may be the result of an underlying problem of habit learning rather than an initial problem with attention per se. This has yet to be tested directly in HD mice but procedural learning (spatial or non-spatial), which forms the basis of habit acquisition, has been found to be disrupted in a number of HD mouse lines using a simple swim tank test. Mice are trained to swim to one or other end of a swim tank using egocentric cues, for example following a specific non-spatial cue such as a light. This swim tank procedural learning protocol measures the ability of the mice to acquire and maintain a simple rule and is often followed by a reversal procedure. Many mouse lines demonstrate deficits in this type of task including the R6/1 and R6/2 lines, the YAC128 and BACHD lines, the zQ175 line (Heikkinen et al. 2012; Menalled et al. 2012; Brooks et al. 2012d; Abada et al. 2013; Ciamei and Morton 2009; Van Raamsdonk et al. 2005b; Lione et al. 1999).

Despite the fact that short-term memory deficits are routinely found to be one of the most robust of the cognitive deficits observed in HD patients (Snowden et al. 2002; Solomon et al. 2007; Stout et al. 2012; Tabrizi et al. 2013), there are few studies in HD mouse lines. Working memory is encoded in corticostriatal circuits at the synapses between descending glutamatergic afferent fibres from the cortex and recipient MSNs and cholinergic inter-neurons, which can undergo both long term-potentiation (LTP) and long-term depression (LTD) (Calabresi et al. 1992a, b). LTD was found to be dependent on the co-activation of dopamine D1 and D2 receptors and on the metabotropic mGluR1 receptors (Calabresi et al. 1992a; Gubellini et al. 2001, 2004), whereas LTP was found to be dependent on NMDA glutamine receptors and dopamine D1 and D5 receptors (Schotanus and Chergui 2008; Kerr and Wickens 2001), with the activation of cholinergic M1 and deactivation of M2 receptors also influential (Calabresi et al. 1992b, 1999). Whilst several other neurotransmitters are able to influence corticostriatal plasticity including CB1 receptors (Gerdeman et al. 2002), it is clear that the reported changes in dopamine and glutamate transmission in the mouse models and the HD brain (Andre et al. 2011; Callahan and Abercrombie 2011; Glass et al. 2000), may be responsible for the early onset of working memory deficits. Spatial and non-spatial working memory deficits, sometimes defined as the ability to alternate, have been reported in a number of mouse lines including the R6/2, R6/1 and HdhQ92 mice (Lione et al. 1999; Nithianantharajah et al. 2008; Pang et al. 2006; Ruskin et al. 2011; Trueman et al. 2009), as have deficits in recognition memory using objects as discriminants (Doria et al. 2013; Giralt et al. 2011b), and in the R6/2 mouse LTP was found to be reduced in dopamine sensitive cells of the dorsal striatum (Kung et al. 2007). It should also be noted that plastic changes can be achieved through increasing synaptic throughput (Daoudal and Debanne 2003), providing a mechanism for subtle changes within the striatum where increased neuronal activity has been reported in a number of mouse lines (Joshi et al. 2009; Heikkinen et al. 2012;

Dvorzhak et al. 2013). Whilst the alternation type of task probes corticostriatal function, the object recognition task is mediated by the hippocampus and associated structures including the entorhinal and perirhinal cortices (Tatro et al. 2013; Soontornniyomkij et al. 2010, 2012; Albasser et al. 2010; Le Merrer et al. 2013), suggesting that deficits in this test may not be representative of the earlier stages of HD pathology.

With regard to other indicators of hippocampal function, spatial learning using the Morris water maze has been examined in the HD mouse lines. In addition to the presence of inclusion pathology (Bayram-Weston et al. 2012b, d; Murphy et al. 2000), HD mouse lines exhibit a broad range of hippocampal abnormalities including NMDA receptor changes, BDNF down regulation and reductions in neurogenesis (Giralt et al. 2009, 2011b, 2012; Murphy et al. 2000; Rattray et al. 2013; Ransome and Hannan 2013; Orvoen et al. 2012), which may contribute to spatial learning deficits. In addition, a degree of functional compensation has been found to exist between the hippocampus and striatum in both HD and the R6/2 mouse models (Ciamei and Morton 2009; Voermans et al. 2004), but anatomical studies suggest the major functional innervation of the striatum is to ventral striatal regions from the CA1 region in the hippocampus (Groenewegen and Trimble 2007), and not to dorsal striatal regions that would be expected to contribute to learning. Spatial learning deficits have been found in the R6/1 and R6/2 mice, HdhQ150 line and YAC128 mice (Brooks et al. 2012a, b, e; Lione et al. 1999; Murphy et al. 2000).

In summary, cognitive deficits in mouse models of HD are varied but generally consistent with what we would expect from the disease state in humans. The obvious difference is the involvement of hippocampal pathology that can be pronounced in some models which detract from the face validity of the disease state, especially as it can be prominent from an early age in some mouse lines.

6 The Neurobiology of Behavioural Dysfunction in Huntington's Disease Mouse Lines

There is significant evidence that people with HD have a broad range of psychiatric and behavioural problems. These include a high preponderance of depression (Van Duijn et al. 2007, 2008), but also anxiety, irritability impulsiveness and associated maladies, and rarely overt schizophrenia-like psychosis (Duff et al. 2007; Kingma et al. 2008; Marshall et al. 2007; Van Duijn et al. 2007, 2008). Probably the single most robust deficit within the behavioural domain is apathy (Baudic et al. 2006; Reedeker et al. 2011; Vaccarino et al. 2011; Van Duijn et al. 2010), which can increase with disease progression and supercede other early stage behavioural abnormalities such as irritability (Thompson et al. 2012), which is also a significant marker of early stage disease (Kloppel et al. 2010; Paulsen et al. 2001; Reedeker et al. 2012; Thompson et al. 2002, 2012).

In mice, many of these behavioural abnormalities are difficult to test in part due to the species differences in levels of consciousness, and the inability to use verbal and questionnaire means of testing. Thus, we can conceive that mice may be able to experience hallucinations for example, yet the challenge lies in how to probe and test these functions through observation and measurement of non-verbal behaviour. Nevertheless, we can operationally define the aspects of psychiatric dysfunction in terms of corresponding behavioural readouts. For example, anhedonia (reduced perception of reward) in humans may correlate in part with reduced motivation to work for a reward in mice, and it is possible to design tests of decision and choice behaviour that can reflect aspects of impulsivity. There is vast literature on these aspects of behaviour that focuses on ventral striatal regions and implicates monoaminergic substrates, dopamine especially (Fig. 1c), as underlying these behavioural phenomena (Bari and Robbins 2013; Dalley et al. 2008; Furuyashiki and Deguchi 2012; Gorwood 2008; Nestler and Carlezon 2006; Robbins et al. 2012). In addition, the HD mouse literature that describes striatal abnormalities in these neurotransmitter systems and circuits (see above) suggests that tests to probe these deficits will be of great value. These operationally defined aspects of rodent behaviour allow us to develop tests that can then be validated pharmacologically with existing compounds previously tested against the human malady.

In HD mouse lines, very few studies exist that have attempted to investigate these phenomena and where they do exist, the data are often difficult to interpret due to operational issues. The classical pharmacological test of depression-like behaviour is the Porsolt forced swim test, where animals are placed in a bowl of water and allowed to swim. The latency to inactivation is taken as a measure of "behavioural despair", akin to learned helplessness in people. The problem with this approach is that if the HD mice are motorically impaired, they are likely to swim less than their wildtype littermates: so what exactly is being measured, behavioural despair or swimming proficiency? As some mouse lines such as the YAC128, BACHD and R6 lines demonstrate early motor deficits, can these lines really be considered to be exhibiting behavioural despair, especially since swimming behaviour is a good measure of motor dysfunction? Several HD mouse lines have been found to be impaired on the forced swim type of task including the BACHD, YAC128, R6/1 and R6/2 mice, N171-82Q and the HdhQ111 lines (Chiu et al. 2011; Hult et al. 2013; Orvoen et al. 2012; Renoir et al. 2011, 2012, 2013), but in most cases these results have been validated through the use of secondary tests of depression-like behaviour or with pharmacological probes (Chiu et al. 2011; Hult et al. 2013; Orvoen et al. 2012; Renoir et al. 2011, 2012, 2013). In one study the use of a standard selective serotonin reuptake inhibitor (SSRI) sertraline failed to reverse these effects suggesting that the depression-like observations were not mediated by serotonin in the BACHD mouse line (Hult et al. 2013). In contrast, studies by another group (Renoir et al. 2011, 2012, 2013) found that sertraline action particularly in the hippocampus was responsible for the improvement in the depression-like phenotype in the R6/1 mouse line, and that environmental enrichment improved an anxiety-like phenotype independently of these effects.

In other studies with sertraline, retardation of disease-induced anatomical changes and motor readouts has been reported, with the authors suggesting that these effects were BDNF and/or neurogenesis mediated (Cheng et al. 2011; Duan et al. 2008; Peng et al. 2008). However, in these studies, the authors did not examine the depression-like phenotype and the precise mechanism of action of sertraline remains to be elucidated. The anxiety phenotype was also examined in many of the studies cited previously and others (Abada et al. 2013; Chiu et al. 2011; Hickey et al. 2008; Hult et al. 2013; Orvoen et al. 2012; Renoir et al. 2013), but these deficits are rarely targeted in pharmacological trials or with pharmacological probes making a dissection of neuronal substrates difficult.

Sleep disturbance in HD is gaining increased attention and has been found in HD patients and mouse models of the disease (Goodman and Barker 2010; Goodman et al. 2011; Kudo et al. 2011; Maywood et al. 2010; Pignatelli et al. 2012; Van Wamelen et al. 2013; Williams et al. 2011; Wood et al. 2013; Morton et al. 2005). In the HD mice there are clear disturbances of circadian rhythms (Maywood et al. 2010; Kudo et al. 2011; Wood et al. 2013; Morton et al. 2005), and abnormalities in hypothalamic orexin (important in wakefulness) containing cells (Petersen et al. 2005; Williams et al. 2011), although most human studies (Baumann et al. 2006; Gaus et al. 2005; Meier et al. 2005), but not all (Roos and Aziz 2007), demonstrate normal orexin-A levels. Experiments have been conducted to correct the aberrant rhythmicity in the R6/2 mouse with some therapeutic benefit being derived (Maywood et al. 2010). These studies are still in the early stages of development though as sleep research is difficult to conduct even in a controlled environment, but the study of circadian rhythms has promise as a potential non-invasive therapeutic intervention.

The study of apathy and irritability is difficult in mice due to lack of clear operational definitions and validated tasks for mice. However, the research community is now beginning to address these issues and increasingly novel tests that focus on behavioural abnormalities, such as impulsivity, are becoming more prevalent but have yet to be published in the HD field. As a result, the study of the neurobiology that underlies the behavioural phenotypes in the HD mouse lines is still in its infancy, but there are considerable efforts being made to advance this aspect of research.

7 General Conclusions

At present, the development of behavioural probes to accurately gauge these changes in rodents is crucial to the advancement of the field and is ongoing in several laboratories. But we have good probes of motor and cognitive symptoms for the mouse models, allowing trials in a broad range of interventions to be run with some success. However, it is these same trials that highlight the differences between the mouse lines and the human condition in that we still have no effective treatment for HD, despite a wide range of compounds demonstrating positive

results in mouse models. With the advancement of MRI and other techniques and the development of more sophisticated clinical assessment with which to study HD, our understanding of the neurobiology of the disease and how it impacts on specific functional readouts has improved greatly. These techniques have also significantly improved our knowledge of the neurobiology of the mouse lines and increasingly this knowledge is being used to directly probe the multi-layered effects of novel therapeutic approaches in a highly targeted way, allowing us to match disease retardation with biological change. The challenge at the time of writing is to exploit the early manifestation of psychiatric (behavioural) symptoms to provide early disease readouts, which will allow the efficacy of early therapeutic interventions to be assessed. This is continuing at some pace and ultimately will provide a unified (motor, cognitive, behavioural) therapeutic platform more consistent with the human condition and the integrated biology of the mammalian organism.

References

Abada YS, Schreiber R, Ellenbroek B (2013) Motor, emotional and cognitive deficits in adult BACHD mice: a model for Huntington's disease. Behav Brain Res 238:243–251

Agnoli L, Mainolfi P, Invernizzi RW, Carli M (2013) Dopamine D1-like and D2-like receptors in the dorsal striatum control different aspects of attentional performance in the five-choice serial reaction time task under a condition of increased activity of corticostriatal inputs. Neuropsychopharmacology 38:701–714

Albasser MM, Poirier GL, Aggleton JP (2010) Qualitatively different modes of perirhinal-hippocampal engagement when rats explore novel vs. familiar objects as revealed by c-Fos imaging. Eur J Neurosci 31:134–147

Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119–146

Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381

Allen KL, Waldvogel HJ, Glass M, Faull RL (2009) Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. J Chem Neuroanat 37:266–281

Andre VM, Cepeda C, Venegas A, Gomez Y, Levine MS (2006) Altered cortical glutamate receptor function in the R6/2 model of Huntington's disease. J Neurophysiol 95:2108–2119

Andre VM, Fisher YE, Levine MS (2011) Altered balance of activity in the striatal direct and indirect pathways in mouse models of Huntington's disease. Front Syst Neurosci 5:46

Ariano MA, Aronin N, DiFiglia M, Tagle DA, Sibley DR, Leavitt BR, Hayden MR, Levine MS (2002) Striatal neurochemical changes in transgenic models of Huntington's disease. J Neurosci Res 68:716–729

Aylward EH, Nopoulos PC, Ross CA, Langbehn DR, Pierson RK, Mills JA, Johnson HJ, Magnotta VA, Juhl AR, Paulsen JS (2011) Longitudinal change in regional brain volumes in prodromal Huntington disease. J Neurol Neurosurg Psychiatry 82:405–410

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250–4258

Bari A, Robbins TW (2013) Inhibition and impulsivity: behavioral and neural basis of response control. Prog Neurobiol 108:44–79

Bari M, Battista N, Valenza M, Mastrangelo N, Malaponti M, Catanzaro G, Centonze D, Finazzi-Agro A, Cattaneo E, Maccarrone M (2013) In vitro and in vivo models of Huntington's disease show alterations in the endocannabinoid system. FEBS J 280(14):3376–88

Baudic S, Maison P, Dolbeau G, Boisse MF, Bartolomeo P, Dalla BG, Traykov L, Bachoud-Levi AC (2006) Cognitive impairment related to apathy in early Huntington's disease. Dement Geriatr Cogn Disord 21:316–321

Baumann CR, Hersberger M, Bassetti CL (2006) Hypocretin-1 (orexin A) levels are normal in Huntington's disease. J Neurol 253:1232–1233

Bayram-Weston Z, Jones L, Dunnett SB, Brooks SP (2012a) Light and electron microscopic characterization of the evolution of cellular pathology in HdhQ92 Huntington's disease knock-in mice. Brain Res Bull 88:171–181

Bayram-Weston Z, Jones L, Dunnett SB, Brooks SP (2012b) Light and electron microscopic characterization of the evolution of cellular pathology in the R6/1 Huntington's disease transgenic mice. Brain Res Bull 88:104–112

Bayram-Weston Z, Jones L, Dunnett SB, Brooks SP (2012c) Light and electron microscopic characterization of the evolution of cellular pathology in YAC128 Huntington's disease transgenic mice. Brain Res Bull 88:137–147

Bayram-Weston Z, Torres EM, Jones L, Dunnett SB, Brooks SP (2012d) Light and electron microscopic characterization of the evolution of cellular pathology in the Hdh(CAG)150 Huntington's disease knock-in mouse. Brain Res Bull 88:189–198

Benn CL, Landles C, Li H, Strand AD, Woodman B, Sathasivam K, Li SH, Ghazi-Noori S, Hockly E, Faruque SM, Cha JH, Sharpe PT, Olson JM, Li XJ, Bates GP (2005) Contribution of nuclear and extranuclear PolyQ to neurological phenotypes in mouse models of Huntington's disease. Hum Mol Genet 14:3065–3078

Bird ED, Spokes EG, Iversen LL (1980) Dopamine and noradrenaline in post-mortem brain in Huntington's disease and schizophrenic illness. Acta Psychiatr Scand Suppl 280:63–73

Bissonette GB, Martins GJ, Franz TM, Harper ES, Schoenbaum G, Powell EM (2008) Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. J Neurosci 28:11124–11130

Bjorklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci 30:194–202

Bjorkqvist M, Fex M, Renstrom E, Wierup N, Petersen A, Gil J, Bacos K, Popovic N, Li JY, Sundler F, Brundin P, Mulder H (2005) The R6/2 transgenic mouse model of Huntington's disease develops diabetes due to deficient beta-cell mass and exocytosis. Hum Mol Genet 14:565–574

Braak H, Braak E (1992a) Allocortical involvement in Huntington's disease. Neuropathol Appl Neurobiol 18:539–547

Braak H, Braak E (1992b) The human entorhinal cortex: normal morphology and lamina-specific pathology in various diseases. Neurosci Res 15:6–31

Brooks S, Higgs G, Janghra N, Jones L, Dunnett SB (2012a) Longitudinal analysis of the behavioural phenotype in YAC128 (C57BL/6 J) Huntington's disease transgenic mice. Brain Res Bull 88:113–120

Brooks S, Higgs G, Jones L, Dunnett SB (2012b) Longitudinal analysis of the behavioural phenotype in Hdh(CAG)150 Huntington's disease knock-in mice. Brain Res Bull 88:182–188

Brooks S, Higgs G, Jones L, Dunnett SB (2012c) Longitudinal analysis of the behavioural phenotype in HdhQ92 Huntington's disease knock-in mice. Brain Res Bull 88:148–155

Brooks SP, Betteridge H, Trueman RC, Jones L, Dunnett SB (2006) Selective extra-dimensional set shifting deficit in a knock-in mouse model of Huntington's disease. Brain Res Bull 69:452–457

Brooks SP, Janghra N, Higgs GV, Bayram-Weston Z, Heuer A, Jones L, Dunnett SB (2012d) Selective cognitive impairment in the YAC128 Huntington's disease mouse. Brain Res Bull 88:121–129

Brooks SP, Janghra N, Workman VL, Bayram-Weston Z, Jones L, Dunnett SB (2012e) Longitudinal analysis of the behavioural phenotype in R6/1 (C57BL/6 J) Huntington's disease transgenic mice. Brain Res Bull 88:94–103

Brooks SP, Jones L, Dunnett SB (2012f) Longitudinal analyses of operant performance on the serial implicit learning task (SILT) in the YAC128 Huntington's disease mouse line. Brain Res Bull 88:130–136

Bussey TJ, Muir JL, Everitt BJ, Robbins TW (1997) Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. Behav Neurosci 111:920–936

Calabresi P, Centonze D, Gubellini P, Bernardi G (1999) Activation of M1-like muscarinic receptors is required for the induction of corticostriatal LTP. Neuropharmacology 38:323–326

Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992a) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:4224–4233

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1992b) Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. Eur J Neurosci 4:929–935

Callahan JW, Abercrombie ED (2011) In vivo Dopamine Efflux is decreased in striatum of both fragment (R6/2) and full-length (YAC128) transgenic mouse models of Huntington's disease. Front Syst Neurosci 5:61

Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, Mengod G, Ernfors P, Alberch J (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 24:7727–7739

Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 19:3248–3257

Cepeda C, Ariano MA, Calvert CR, Flores-Hernandez J, Chandler SH, Leavitt BR, Hayden MR, Levine MS (2001) NMDA receptor function in mouse models of Huntington disease. J Neurosci Res 66:525–539

Chase EA, Tait DS, Brown VJ (2012) Lesions of the orbital prefrontal cortex impair the formation of attentional set in rats. Eur J Neurosci 36:2368–2375

Cheng Y, Peng Q, Hou Z, Aggarwal M, Zhang J, Mori S, Ross CA, Duan W (2011) Structural MRI detects progressive regional brain atrophy and neuroprotective effects in N171-82Q Huntington's disease mouse model. Neuroimage 56:1027–1034

Chiodi V, Uchigashima M, Beggiato S, Ferrante A, Armida M, Martire A, Potenza RL, Ferraro L, Tanganelli S, Watanabe M, Domenici MR, Popoli P (2012) Unbalance of CB1 receptors expressed in GABAergic and glutamatergic neurons in a transgenic mouse model of Huntington's disease. Neurobiol Dis 45:983–991

Chiu CT, Liu G, Leeds P, Chuang DM (2011) Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. Neuropsychopharmacology 36:2406–2421

Chudasama Y, Robbins TW (2006) Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol Psychol 73(1):19–38

Ciamei A, Morton AJ (2009) Progressive imbalance in the interaction between spatial and procedural memory systems in the R6/2 mouse model of Huntington's disease. Neurobiol Learn Mem 92:417–428

Clarke HF, Robbins TW, Roberts AC (2008) Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. J Neurosci 28:10972–10982

Crook ZR, Housman DE (2012) Dysregulation of dopamine receptor D2 as a sensitive measure for Huntington disease pathology in model mice. Proc Natl Acad Sci U S A 109:7487–7492

Cross A, Rossor M (1983) Dopamine D-1 and D-2 receptors in Huntington's disease. Eur J Pharmacol 88:223–229

Cummings DM, Cepeda C, Levine MS (2010) Alterations in striatal synaptic transmission are consistent across genetic mouse models of Huntington's disease. ASN Neuro 2:e00036

Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Behav 90:250–260

Daoudal G, Debanne D (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn Mem 10:456–465

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548

de la Monte SM, Vonsattel JP, Richardson EP Jr (1988) Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. J Neuropathol Exp Neurol 47:516–525

de Tommaso M, Serpino C, Sciruicchio V (2011) Management of Huntington's disease: role of tetrabenazine. Ther Clin Risk Manag 7:123–129

Deckel AW, Tang V, Nuttal D, Gary K, Elder R (2002) Altered neuronal nitric oxide synthase expression contributes to disease progression in Huntington's disease transgenic mice. Brain Res 939:76–86

Dey ND, Bombard MC, Roland BP, Davidson S, Lu M, Rossignol J, Sandstrom MI, Skeel RL, Lescaudron L, Dunbar GL (2010) Genetically engineered mesenchymal stem cells reduce behavioral deficits in the YAC 128 mouse model of Huntington's disease. Behav Brain Res 214:193–200

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993

Divac I, Markowitsch HJ, Pritzel M (1978) Behavioral and anatomical consequences of small intrastriatal injections of kainic acid in the rat. Brain Res 151:523–532

Divac I, Rosvold HE, Szwarcbart MK (1967) Behavioral effects of selective ablation of the caudate nucleus. J Comp Physiol Psychol 63:184–190

Doria J, Silva F, de Souza J, Vieira L, Carvalho T, Reis H, Pereira G, Dobransky T, Ribeiro F (2013) Metabotropic glutamate receptor 5 positive allosteric modulators are neuroprotective in a mouse model of Huntington's disease. Br J Pharmacol 169:909–921

Dowie MJ, Bradshaw HB, Howard ML, Nicholson LF, Faull RL, Hannan AJ, Glass M (2009) Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic mouse model of Huntington's disease. Neuroscience 163:456–465

Dowie MJ, Howard ML, Nicholson LF, Faull RL, Hannan AJ, Glass M (2010) Behavioural and molecular consequences of chronic cannabinoid treatment in Huntington's disease transgenic mice. Neuroscience 170:324–336

Draganski B, Kherif F, Kloppel S, Cook PA, Alexander DC, Parker GJ, Deichmann R, Ashburner J, Frackowiak RS (2008) Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. J Neurosci 28:7143–7152

Duan W, Peng Q, Masuda N, Ford E, Tryggestad E, Ladenheim B, Zhao M, Cadet JL, Wong J, Ross CA (2008) Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease. Neurobiol Dis 30:312–322

Duff K, Paulsen JS, Beglinger LJ, Langbehn DR, Stout JC (2007) Psychiatric symptoms in Huntington's disease before diagnosis: the predict-HD study. Biol Psychiatry 62:1341–1346

Dvorzhak A, Semtner M, Faber DS, Grantyn R (2013) Tonic mGluR5/CB1-dependent suppression of inhibition as a pathophysiological hallmark in the striatum of mice carrying a mutant form of huntingtin. J Physiol 591:1145–1166

Farrar AM, Callahan JW, Abercrombie ED (2011) Reduced striatal acetylcholine efflux in the R6/2 mouse model of Huntington's disease: an examination of the role of altered inhibitory and excitatory mechanisms. Exp Neurol 232:119–125

Filloux F, Wagster MV, Folstein S, Price DL, Hedreen JC, Dawson TM, Wamsley JK (1990) Nigral dopamine type-1 receptors are reduced in Huntington's disease: a postmortem

autoradiographic study using [3H]SCH 23390 and correlation with [3H]forskolin binding. Exp Neurol 110:219–227
Floresco SB, Block AE, Tse MT (2008) Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav Brain Res 190:85–96
Furr A, Lapiz-Bluhm MD, Morilak DA (2012) 5-HT2A receptors in the orbitofrontal cortex facilitate reversal learning and contribute to the beneficial cognitive effects of chronic citalopram treatment in rats. Int J Neuropsychopharmacol 15:1295–1305
Furuyashiki T, Deguchi Y (2012) Roles of altered striatal function in major depression. Brain Nerve 64:919–926
Gaus SE, Lin L, Mignot E (2005) CSF hypocretin levels are normal in Huntington's disease patients. Sleep 28:1607–1608
Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451
Gibson HE, Reim K, Brose N, Morton AJ, Jones S (2005) A similar impairment in CA3 mossy fibre LTP in the R6/2 mouse model of Huntington's disease and in the complexin II knockout mouse. Eur J Neurosci 22:1701–1712
Giralt A, Carreton O, Lao-Peregrin C, Martin ED, Alberch J (2011a) Conditional BDNF release under pathological conditions improves Huntington's disease pathology by delaying neuronal dysfunction. Mol Neurodegener 6:71
Giralt A, Puigdellivol M, Carreton O, Paoletti P, Valero J, Parra-Damas A, Saura CA, Alberch J, Gines S (2012) Long-term memory deficits in Huntington's disease are associated with reduced CBP histone acetylase activity. Hum Mol Genet 21:1203–1216
Giralt A, Rodrigo T, Martin ED, Gonzalez JR, Mila M, Cena V, Dierssen M, Canals JM, Alberch J (2009) Brain-derived neurotrophic factor modulates the severity of cognitive alterations induced by mutant huntingtin: involvement of phospholipaseC gamma activity and glutamate receptor expression. Neuroscience 158:1234–1250
Giralt A, Saavedra A, Carreton O, Xifro X, Alberch J, Perez-Navarro E (2011b) Increased PKA signaling disrupts recognition memory and spatial memory: role in Huntington's disease. Hum Mol Genet 20:4232–4247
Glass M, Dragunow M, Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97:505–519
Glass M, Faull RL, Dragunow M (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. Neuroscience 56:523–527
Glass M, Van Dellen A, Blakemore C, Hannan AJ, Faull RL (2004) Delayed onset of Huntington's disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. Neuroscience 123:207–212
Gomez-Tortosa E, MacDonald ME, Friend JC, Taylor SA, Weiler LJ, Cupples LA, Srinidhi J, Gusella JF, Bird ED, Vonsattel JP, Myers RH (2001) Quantitative neuropathological changes in presymptomatic Huntington's disease. Ann Neurol 49:29–34
Goodman AO, Barker RA (2010) How vital is sleep in Huntington's disease? J Neurol 257:882–897
Goodman AO, Rogers L, Pilsworth S, McAllister CJ, Shneerson JM, Morton AJ, Barker RA (2011) Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. Curr Neurol Neurosci Rep 11:211–217
Gorwood P (2008) Neurobiological mechanisms of anhedonia. Dialogues Clin Neurosci 10:291–299
Graveland GA, Williams RS, DiFiglia M (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. Science 227:770–773
Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195

Griffioen KJ, Wan R, Brown TR, Okun E, Camandola S, Mughal MR, Phillips TM, Mattson MP (2012) Aberrant heart rate and brainstem brain-derived neurotrophic factor (BDNF) signaling in a mouse model of Huntington's disease. Neurobiol Aging 33:1481–1485

Groenewegen HJ, Trimble M (2007) The ventral striatum as an interface between the limbic and motor systems. CNS Spectr 12:887–892

Gubellini P, Pisani A, Centonze D, Bernardi G, Calabresi P (2004) Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases. Prog Neurobiol 74:271–300

Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, Calabresi P (2001) Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology 40:839–846

Hampshire A, Chaudhry AM, Owen AM, Roberts AC (2012) Dissociable roles for lateral orbitofrontal cortex and lateral prefrontal cortex during preference driven reversal learning. Neuroimage 59:4102–4112

Hansson O, Guatteo E, Mercuri NB, Bernardi G, Li XJ, Castilho RF, Brundin P (2001) Resistance to NMDA toxicity correlates with appearance of nuclear inclusions, behavioural deficits and changes in calcium homeostasis in mice transgenic for exon 1 of the huntington gene. Eur J Neurosci 14:1492–1504

Heikkinen T, Lehtimaki K, Vartiainen N, Puolivali J, Hendricks SJ, Glaser JR, Bradaia A, Wadel K, Touller C, Kontkanen O, Yrjanheikki JM, Buisson B, Howland D, Beaumont V, Munoz-Sanjuan I, Park LC (2012) Characterization of neurophysiological and behavioral changes, MRI brain volumetry and 1H MRS in zQ175 knock-in mouse model of Huntington's disease. PLoS ONE 7:e50717

Heinsen H, Rub U, Bauer M, Ulmar G, Bethke B, Schuler M, Bocker F, Eisenmenger W, Gotz M, Korr H, Schmitz C (1999) Nerve cell loss in the thalamic mediodorsal nucleus in Huntington's disease. Acta Neuropathol 97:613–622

Heinsen H, Strik M, Bauer M, Luther K, Ulmar G, Gangnus D, Jungkunz G, Eisenmenger W, Gotz M (1994) Cortical and striatal neurone number in Huntington's disease. Acta Neuropathol 88:320–333

Heng MY, Tallaksen-Greene SJ, Detloff PJ, Albin RL (2007) Longitudinal evaluation of the Hdh(CAG)150 knock-in murine model of Huntington's disease. J Neurosci 27:8989–8998

Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, Chesselet MF (2008) Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. Neuroscience 157:280–295

Hickey MA, Reynolds GP, Morton AJ (2002) The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. J Neurochem 81:46–59

Hickey MA, Zhu C, Medvedeva V, Lerner RP, Patassini S, Franich NR, Maiti P, Frautschy SA, Zeitlin S, Levine MS, Chesselet MF (2012) Improvement of neuropathology and transcriptional deficits in CAG 140 knock-in mice supports a beneficial effect of dietary curcumin in Huntington's disease. Mol Neurodegener 7:12

Hult LS, Nilsson N, Soylu R, Kirik D, Petersen A (2013) Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington's disease. Hum Mol Genet 22(17):3485–3497

Hurlbert MS, Zhou W, Wasmeier C, Kaddis FG, Hutton JC, Freed CR (1999) Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. Diabetes 48:649–651

Jiang M, Peng Q, Liu X, Jin J, Hou Z, Zhang J, Mori S, Ross CA, Ye K, Duan W (2013) Small-molecule TrkB receptor agonists improve motor function and extend survival in a mouse model of Huntington's disease. Hum Mol Genet 22(12):2462–2470

Josefsen K, Nielsen MD, Jorgensen KH, Bock T, Norremolle A, Sorensen SA, Naver B, Hasholt L (2008) Impaired glucose tolerance in the R6/1 transgenic mouse model of Huntington's disease. J Neuroendocrinol 20:165–172

Joshi PR, Wu NP, Andre VM, Cummings DM, Cepeda C, Joyce JA, Carroll JB, Leavitt BR, Hayden MR, Levine MS, Bamford NS (2009) Age-dependent alterations of corticostriatal activity in the YAC128 mouse model of Huntington disease. J Neurosci 29:2414–2427

Josiassen RC, Curry LM, Mancall EL (1983) Development of neuropsychological deficits in Huntington's disease. Arch Neurol 40:791–796

Kerr JN, Wickens JR (2001) Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J Neurophysiol 85:117–124

Kim J, Ragozzino ME (2005) The involvement of the orbitofrontal cortex in learning under changing task contingencies. Neurobiol Learn Mem 83:125–133

Kingma EM, Van Duijn E, Timman R, Van der Mast RC, Roos RA (2008) Behavioural problems in Huntington's disease using the problem behaviours assessment. Gen Hosp Psychiatry 30:155–161

Kiriazis H, Jennings NL, Davern P, Lambert G, Su Y, Pang T, Du X, La Greca L, Head GA, Hannan AJ, Du XJ (2012) Neurocardiac dysregulation and neurogenic arrhythmias in a transgenic mouse model of Huntington's disease. J Physiol 590:5845–5860

Klanker M, Post G, Joosten R, Feenstra M, Denys D (2013) Deep brain stimulation in the lateral orbitofrontal cortex impairs spatial reversal learning. Behav Brain Res 245:7–12

Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet MF, Levine MS (2001) Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice. J Neurophysiol 86:2667–2677

Kloppel S, Stonnington CM, Petrovic P, Mobbs D, Tuscher O, Craufurd D, Tabrizi SJ, Frackowiak RS (2010) Irritability in pre-clinical Huntington's disease. Neuropsychologia 48:549–557

Kudo T, Schroeder A, Loh DH, Kuljis D, Jordan MC, Roos KP, Colwell CS (2011) Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. Exp Neurol 228:80–90

Kung VW, Hassam R, Morton AJ, Jones S (2007) Dopamine-dependent long term potentiation in the dorsal striatum is reduced in the R6/2 mouse model of Huntington's disease. Neuroscience 146:1571–1580

Kurlan R, Goldblatt D, Zaczek R, Jeffries K, Irvine C, Coyle J, Shoulson I (1988) Cerebrospinal fluid homovanillic acid and parkinsonism in Huntington's disease. Ann Neurol 24:282–284

Labuschagne I, Jones R, Callaghan J, Whitehead D, Dumas EM, Say MJ, Hart EP, Justo D, Coleman A, Dar Santos RC, Frost C, Craufurd D, Tabrizi SJ, Stout JC (2013) Emotional face recognition deficits and medication effects in pre-manifest through stage-II Huntington's disease. Psychiatry Res 207:118–126

Lawrence AD, Hodges JR, Rosser AE, Kershaw A, Ffrench-Constant C, Rubinsztein DC, Robbins TW, Sahakian BJ (1998) Evidence for specific cognitive deficits in preclinical Huntington's disease. Brain 121(Pt 7):1329–1341

Lawrence AD, Sahakian BJ, Hodges JR, Rosser AE, Lange KW, Robbins TW (1996) Executive and mnemonic functions in early Huntington's disease. Brain 119(Pt 5):1633–1645

Lawrence AD, Sahakian BJ, Rogers RD, Hodge JR, Robbins TW (1999) Discrimination, reversal, and shift learning in Huntington's disease: mechanisms of impaired response selection. Neuropsychologia 37:1359–1374

Lawrence AD, Watkins LH, Sahakian BJ, Hodges JR, Robbins TW (2000) Visual object and visuospatial cognition in Huntington's disease: implications for information processing in corticostriatal circuits. Brain 123(Pt 7):1349–1364

Le Merrer J, Rezai X, Scherrer G, Becker JA, Kieffer BL (2013) Impaired hippocampus-dependent and facilitated striatum-dependent behaviors in mice lacking the delta opioid receptor. Neuropsychopharmacology 38:1050–1059

Lehericy S, Ducros M, Van de Moortele PF, Francois C, Thivard L, Poupon C, Swindale N, Ugurbil K, Kim DS (2004) Diffusion tensor fiber tracking shows distinct corticostriatal circuits in humans. Ann Neurol 55:522–529

Lerner RP, Trejo Martinez LC, Zhu C, Chesselet MF, Hickey MA (2012) Striatal atrophy and dendritic alterations in a knock-in mouse model of Huntington's disease. Brain Res Bull 87:571–578

Levine MS, Klapstein GJ, Koppel A, Gruen E, Cepeda C, Vargas ME, Jokel ES, Carpenter EM, Zanjani H, Hurst RS, Efstratiadis A, Zeitlin S, Chesselet MF (1999) Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. J Neurosci Res 58:515–532

Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10:137–144

Lindgren HS, Wickens R, Tait DS, Brown VJ, Dunnett SB (2013) Lesions of the dorsomedial striatum impair formation of attentional set in rats. Neuropharmacology 71:148–153

Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB (1999) Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. J Neurosci 19:10428–10437

Liot G, Zala D, Pla P, Mottet G, Piel M, Saudou F (2013) Mutant Huntingtin alters retrograde transport of TrkB receptors in striatal dendrites. J Neurosci 33:6298–6309

Mandal M, Wei J, Zhong P, Cheng J, Duffney LJ, Liu W, Yuen EY, Twelvetrees AE, Li S, Li XJ, Kittler JT, Yan Z (2011) Impaired alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking and function by mutant huntingtin. J Biol Chem 286:33719–33728

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506

Mann DM (1989) Subcortical afferent projection systems in Huntington's chorea. Acta Neuropathol 78:551–554

Marshall J, White K, Weaver M, Flury WL, Hui S, Stout JC, Johnson SA, Beristain X, Gray J, Wojcieszek J, Foroud T (2007) Specific psychiatric manifestations among preclinical Huntington disease mutation carriers. Arch Neurol 64:116–121

Martin B, Golden E, Carlson OD, Pistell P, Zhou J, Kim W, Frank BP, Thomas S, Chadwick WA, Greig NH, Bates GP, Sathasivam K, Bernier M, Maudsley S, Mattson MP, Egan JM (2009) Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. Diabetes 58:318–328

Maywood ES, Fraenkel E, McAllister CJ, Wood N, Reddy AB, Hastings MH, Morton AJ (2010) Disruption of peripheral circadian timekeeping in a mouse model of Huntington's disease and its restoration by temporally scheduled feeding. J Neurosci 30:10199–10204

McCool MF, Patel S, Talati R, Ragozzino ME (2008) Differential involvement of M1-type and M4-type muscarinic cholinergic receptors in the dorsomedial striatum in task switching. Neurobiol Learn Mem 89:114–124

Meier A, Mollenhauer B, Cohrs S, Rodenbeck A, Jordan W, Meller J, Otto M (2005) Normal hypocretin-1 (orexin-A) levels in the cerebrospinal fluid of patients with Huntington's disease. Brain Res 1063:201–203

Menalled L, El Khodor BF, Patry M, Suarez-Farinas M, Orenstein SJ, Zahasky B, Leahy C, Wheeler V, Yang XW, MacDonald M, Morton AJ, Bates G, Leeds J, Park L, Howland D, Signer E, Tobin A, Brunner D (2009) Systematic behavioral evaluation of Huntington's disease transgenic and knock-in mouse models. Neurobiol Dis 35:319–336

Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, Ruiz M, Mushlin R, Alosio W, McConnell K, Connor D, Murphy C, Oakeshott S, Kwan M, Beltran J, Ghavami A, Brunner D, Park LC, Ramboz S, Howland D (2012) Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. PLoS ONE 7:e49838

Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF (2003) Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J Comp Neurol 465:11–26

Mievis S, Blum D, Ledent C (2011) Worsening of Huntington disease phenotype in CB1 receptor knockout mice. Neurobiol Dis 42:524–529

Miller J, Arrasate M, Shaby BA, Mitra S, Masliah E, Finkbeiner S (2010) Quantitative relationships between huntingtin levels, polyglutamine length, inclusion body formation, and neuronal death provide novel insight into huntington's disease molecular pathogenesis. J Neurosci 30:10541–10550

Milnerwood AJ, Raymond LA (2007) Corticostriatal synaptic function in mouse models of Huntington's disease: early effects of huntingtin repeat length and protein load. J Physiol 585:817–831

Mochel F, Durant B, Durr A, Schiffmann R (2011) Altered dopamine and serotonin metabolism in motorically asymptomatic R6/2 mice. PLoS ONE 6:e18336

Moffitt H, McPhail GD, Woodman B, Hobbs C, Bates GP (2009) Formation of polyglutamine inclusions in a wide range of non-CNS tissues in the HdhQ150 knock-in mouse model of Huntington's disease. PLoS ONE 4:e8025

Morton AJ, Lagan MA, Skepper JN, Dunnett SB (2000) Progressive formation of inclusions in the striatum and hippocampus of mice transgenic for the human Huntington's disease mutation. J Neurocytol 29:679–702

Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES (2005) Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J Neurosci 25:157–163

Muir JL, Page KJ, Sirinathsinghji DJ, Robbins TW, Everitt BJ (1993) Excitotoxic lesions of basal forebrain cholinergic neurons: effects on learning, memory and attention. Behav Brain Res 57:123–131

Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (2000) Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. J Neurosci 20:5115–5123

Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, Richardson EP Jr, Bird ED (1991) Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. J Neuropathol Exp Neurol 50:729–742

Nestler EJ, Carlezon WA Jr (2006) The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 59:1151–1159

Nithianantharajah J, Barkus C, Murphy M, Hannan AJ (2008) Gene-environment interactions modulating cognitive function and molecular correlates of synaptic plasticity in Huntington's disease transgenic mice. Neurobiol Dis 29:490–504

Norflus F, Nanje A, Gutekunst CA, Shi G, Cohen J, Bejarano M, Fox J, Ferrante RJ, Hersch SM (2004) Anti-inflammatory treatment with acetylsalicylate or rofecoxib is not neuroprotective in Huntington's disease transgenic mice. Neurobiol Dis 17:319–325

Novak MJ, Warren JD, Henley SM, Draganski B, Frackowiak RS, Tabrizi SJ (2012) Altered brain mechanisms of emotion processing in pre-manifest Huntington's disease. Brain 135:1165–1179

Ortiz AN, Kurth BJ, Osterhaus GL, Johnson MA (2010) Dysregulation of intracellular dopamine stores revealed in the R6/2 mouse striatum. J Neurochem 112:755–761

Orvoen S, Pla P, Gardier AM, Saudou F, David DJ (2012) Huntington's disease knock-in male mice show specific anxiety-like behaviour and altered neuronal maturation. Neurosci Lett 507:127–132

Pallier PN, Drew CJ, Morton AJ (2009) The detection and measurement of locomotor deficits in a transgenic mouse model of Huntington's disease are task- and protocol-dependent: influence of non-motor factors on locomotor function. Brain Res Bull 78:347–355

Pang TY, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ (2006) Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in Huntington's disease transgenic mice. Neuroscience 141:569–584

Paulsen JS, Magnotta VA, Mikos AE, Paulson HL, Penziner E, Andreasen NC, Nopoulos PC (2006) Brain structure in preclinical Huntington's disease. Biol Psychiatry 59:57–63

Paulsen JS, Nopoulos PC, Aylward E, Ross CA, Johnson H, Magnotta VA, Juhl A, Pierson RK, Mills J, Langbehn D, Nance M (2010) Striatal and white matter predictors of estimated diagnosis for Huntington disease. Brain Res Bull 82:201–207

Paulsen JS, Ready RE, Hamilton JM, Mega MS, Cummings JL (2001) Neuropsychiatric aspects of Huntington's disease. J Neurol Neurosurg Psychiatry 71:310–314

Peng Q, Masuda N, Jiang M, Li Q, Zhao M, Ross CA, Duan W (2008) The antidepressant sertraline improves the phenotype, promotes neurogenesis and increases BDNF levels in the R6/2 Huntington's disease mouse model. Exp Neurol 210:154–163

Petersen A, Gil J, Maat-Schieman ML, Bjorkqvist M, Tanila H, Araujo IM, Smith R, Popovic N, Wierup N, Norlen P, Li JY, Roos RA, Sundler F, Mulder H, Brundin P (2005) Orexin loss in Huntington's disease. Hum Mol Genet 14:39–47

Pidgeon C, Rickards H (2013) The pathophysiology and pharmacological treatment of Huntington disease. Behav Neurol 26:245–253

Pignatelli M, Lebreton F, Cho YH, Leinekugel X (2012) "Ectopic" theta oscillations and interictal activity during slow-wave state in the R6/1 mouse model of Huntington's disease. Neurobiol Dis 48:409–417

Pisa M, Cyr J (1990) Regionally selective roles of the rat's striatum in modality-specific discrimination learning and forelimb reaching. Behav Brain Res 37:281–292

Pouladi MA, Stanek LM, Xie Y, Franciosi S, Southwell AL, Deng Y, Butland S, Zhang W, Cheng SH, Shihabuddin LS, Hayden MR (2012) Marked differences in neurochemistry and aggregates despite similar behavioural and neuropathological features of Huntington disease in the full-length BACHD and YAC128 mice. Hum Mol Genet 21:2219–2232

Ragozzino ME, Ragozzino KE, Mizumori SJ, Kesner RP (2002) Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. Behav Neurosci 116:105–115

Ransome MI, Hannan AJ (2013) Impaired basal and running-induced hippocampal neurogenesis coincides with reduced Akt signaling in adult R6/1 HD mice. Mol Cell Neurosci 54:93–107

Rattray I, Smith E, Gale R, Matsumoto K, Bates GP, Modo M (2013) Correlations of behavioral deficits with brain pathology assessed through longitudinal MRI and histopathology in the R6/2 mouse model of HD. PLoS ONE 8:e60012

Reddy PH, Charles V, Williams M, Miller G, Whetsell WO Jr, Tagle DA (1999) Transgenic mice expressing mutated full-length HD cDNA: a paradigm for locomotor changes and selective neuronal loss in Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354:1035–1045

Reedeker N, Bouwens JA, Giltay EJ, Le Mair SE, Roos RA, Van der Mast RC, Van Duijn E (2012) Irritability in Huntington's disease. Psychiatry Res 200:813–818

Reedeker N, Bouwens JA, Van Duijn E, Giltay EJ, Roos RA, Van der Mast RC (2011) Incidence, course, and predictors of apathy in Huntington's disease: a two-year prospective study. J Neuropsychiatry Clin Neurosci 23:434–441

Reiner A, Lafferty DC, Wang HB, Del Mar N, Deng YP (2012a) The group 2 metabotropic glutamate receptor agonist LY379268 rescues neuronal, neurochemical and motor abnormalities in R6/2 Huntington's disease mice. Neurobiol Dis 47:75–91

Reiner A, Wang HB, Del Mar N, Sakata K, Yoo W, Deng YP (2012b) BDNF may play a differential role in the protective effect of the mGluR2/3 agonist LY379268 on striatal projection neurons in R6/2 Huntington's disease mice. Brain Res 1473:161–172

Renoir T, Pang TY, Mo C, Chan G, Chevarin C, Lanfumey L, Hannan AJ (2013) Differential effects of early environmental enrichment on emotionality related behaviours in Huntington's disease transgenic mice. J Physiol 591:41–55

Renoir T, Pang TY, Zajac MS, Chan G, Du X, Leang L, Chevarin C, Lanfumey L, Hannan AJ (2012) Treatment of depressive-like behaviour in Huntington's disease mice by chronic sertraline and exercise. Br J Pharmacol 165:1375–1389

Renoir T, Zajac MS, Du X, Pang TY, Leang L, Chevarin C, Lanfumey L, Hannan AJ (2011) Sexually dimorphic serotonergic dysfunction in a mouse model of Huntington's disease and depression. PLoS ONE 6:e22133

Ribchester RR, Thomson D, Wood NI, Hinks T, Gillingwater TH, Wishart TM, Court FA, Morton AJ (2004) Progressive abnormalities in skeletal muscle and neuromuscular junctions of transgenic mice expressing the Huntington's disease mutation. Eur J Neurosci 20:3092–3114

Rising AC, Xu J, Carlson A, Napoli VV, Denovan-Wright EM, Mandel RJ (2011) Longitudinal behavioral, cross-sectional transcriptional and histopathological characterization of a knock-in mouse model of Huntington's disease with 140 CAG repeats. Exp Neurol 228:173–182

Robbins TW, Gillan CM, Smith DG, de Wit S, Ersche KD (2012) Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. Trends Cogn Sci 16:81–91

Roizin L, Stellar S, Willson N, Whittier J, Liu JC (1974) Electron microscope and enzyme studies in cerebral biopsies of Huntington's chorea. Trans Am Neurol Assoc 99:240–243

Roman MJ, Delis DC, Filoteo JV, Demadura TL, Paulsen J, Swerdlow NR, Swenson MR, Salmon D, Butters N, Shults C (1998) Is there a "subcortical" profile of attentional dysfunction? A comparison of patients with Huntington's and Parkinson's diseases on a global-local focused attention task. J Clin Exp Neuropsychol 20:873–884

Roos RA, Aziz NA (2007) Hypocretin-1 and secondary signs in Huntington's disease. Parkinsonism Relat Disord 13(Suppl 3):S387–S390

Rosas HD, Hevelone ND, Zaleta AK, Greve DN, Salat DH, Fischl B (2005) Regional cortical thinning in preclinical Huntington disease and its relationship to cognition. Neurology 65:745–747

Rosas HD, Liu AK, Hersch S, Glessner M, Ferrante RJ, Salat DH, Van der KA, Jenkins BG, Dale AM, Fischl B (2002) Regional and progressive thinning of the cortical ribbon in Huntington's disease. Neurology 58:695–701

Rosvold HE, Delgado JM (1956) The effect on delayed-alternation test performance of stimulating or destroying electrically structures within the frontal lobes of the monkey's brain. J Comp Physiol Psychol 49:365–372

Rosvold HE, Mishkin M, Szwarcbart MK (1958) Effects of subcortical lesions in monkeys on visual-discrimination and single-alternation performance. J Comp Physiol Psychol 51:437–444

Rub U, Hoche F, Brunt ER, Heinsen H, Seidel K, Del Turco D, Paulson HL, Bohl J, von Gall C, Vonsattel JP, Korf HW, den Dunnen WF (2013) Degeneration of the cerebellum in Huntington's disease (HD): possible relevance for the clinical picture and potential gateway to pathological mechanisms of the disease process. Brain Pathol 23:165–177

Ruskin DN, Ross JL, Kawamura M Jr, Ruiz TL, Geiger JD, Masino SA (2011) A ketogenic diet delays weight loss and does not impair working memory or motor function in the R6/2 1 J mouse model of Huntington's disease. Physiol Behav 103:501–507

Samadi P, Boutet A, Rymar VV, Rawal K, Maheux J, Kvann JC, Tomaszewski M, Beaubien F, Cloutier JF, Levesque D, Sadikot AF (2013) Relationship between BDNF expression in major striatal afferents, striatum morphology and motor behavior in the R6/2 mouse model of Huntington's disease. Genes Brain Behav 12:108–124

Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95:55–66

Schilling G, Klevytska A, Tebbenkamp AT, Juenemann K, Cooper J, Gonzales V, Slunt H, Poirer M, Ross CA, Borchelt DR (2007) Characterization of huntingtin pathologic fragments in human Huntington disease, transgenic mice, and cell models. J Neuropathol Exp Neurol 66:313–320

Schmitz C, Rub U, Korr H, Heinsen H (1999) Nerve cell loss in the thalamic mediodorsal nucleus in Huntington's disease. II. Optimization of a stereological estimation procedure. Acta Neuropathol 97:623–628

Schotanus SM, Chergui K (2008) Dopamine D1 receptors and group I metabotropic glutamate receptors contribute to the induction of long-term potentiation in the nucleus accumbens. Neuropharmacology 54:837–844

Slow EJ, Graham RK, Osmand AP, Devon RS, Lu G, Deng Y, Pearson J, Vaid K, Bissada N, Wetzel R, Leavitt BR, Hayden MR (2005) Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. Proc Natl Acad Sci U S A 102:11402–11407

Slow EJ, Van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567

Snowden J, Craufurd D, Griffiths H, Thompson J, Neary D (2001) Longitudinal evaluation of cognitive disorder in Huntington's disease. J Int Neuropsychol Soc 7:33–44

Snowden JS, Austin NA, Sembi S, Thompson JC, Craufurd D, Neary D (2008) Emotion recognition in Huntington's disease and frontotemporal dementia. Neuropsychologia 46:2638–2649

Snowden JS, Craufurd D, Griffiths HL, Neary D (1998) Awareness of involuntary movements in Huntington disease. Arch Neurol 55:801–805

Snowden JS, Craufurd D, Thompson J, Neary D (2002) Psychomotor, executive, and memory function in preclinical Huntington's disease. J Clin Exp Neuropsychol 24:133–145

Snowden JS, Gibbons ZC, Blackshaw A, Doubleday E, Thompson J, Craufurd D, Foster J, Happe F, Neary D (2003) Social cognition in frontotemporal dementia and Huntington's disease. Neuropsychologia 41:688–701

Solomon AC, Stout JC, Johnson SA, Langbehn DR, Aylward EH, Brandt J, Ross CA, Beglinger L, Hayden MR, Kieburtz K, Kayson E, Julian-Baros E, Duff K, Guttman M, Nance M, Oakes D, Shoulson I, Penziner E, Paulsen JS (2007) Verbal episodic memory declines prior to diagnosis in Huntington's disease. Neuropsychologia 45:1767–1776

Soontornniyomkij V, Risbrough VB, Young JW, Soontornniyomkij B, Jeste DV, Achim CL (2012) Increased hippocampal accumulation of autophagosomes predicts short-term recognition memory impairment in aged mice. Age (Dordr) 34:305–316

Soontornniyomkij V, Risbrough VB, Young JW, Wallace CK, Soontornniyomkij B, Jeste DV, Achim CL (2010) Short-term recognition memory impairment is associated with decreased expression of FK506 binding protein 51 in the aged mouse brain. Age (Dordr) 32:309–322

Spampanato J, Gu X, Yang XW, Mody I (2008) Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. Neuroscience 157:606–620

Spires TL, Grote HE, Varshney NK, Cordery PM, Van Dellen A, Blakemore C, Hannan AJ (2004) Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. J Neurosci 24:2270–2276

Spokes EG (1980) Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain 103:179–210

Sprengelmeyer R, Lange H, Homberg V (1995) The pattern of attentional deficits in Huntington's disease. Brain 118(Pt 1):145–152

Starling AJ, Andre VM, Cepeda C, de Lima M, Chandler SH, Levine MS (2005) Alterations in N-methyl-D-aspartate receptor sensitivity and magnesium blockade occur early in development in the R6/2 mouse model of Huntington's disease. J Neurosci Res 82:377–386

Stout JC, Johnson SA (2005) Cognitive impairment and dementia in basal ganglia disorders. Curr Neurol Neurosci Rep 5:355–363

Stout JC, Jones R, Labuschagne I, O'Regan AM, Say MJ, Dumas EM, Queller S, Justo D, Santos RD, Coleman A, Hart EP, Durr A, Leavitt BR, Roos RA, Langbehn DR, Tabrizi SJ, Frost C (2012) Evaluation of longitudinal 12 and 24 month cognitive outcomes in premanifest and early Huntington's disease. J Neurol Neurosurg Psychiatry 83:687–694

Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, Landwehrmeyer GB, Fox NC, Johnson H, Hicks SL, Kennard C, Craufurd D, Frost C, Langbehn DR, Reilmann R, Stout JC

(2011) Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. Lancet Neurol 10:31–42

Tabrizi SJ, Scahill RI, Owen G, Durr A, Leavitt BR, Roos RA, Borowsky B, Landwehrmeyer B, Frost C, Johnson H, Craufurd D, Reilmann R, Stout JC, Langbehn DR (2013) Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol 12(7):637–649

Tatro ET, Risbrough V, Soontornniyomkij B, Young J, Shumaker-Armstrong S, Jeste DV, Achim CL (2013) Short-term recognition memory correlates with regional CNS expression of microRNA-138 in mice. Am J Geriatr Psychiatry 21:461–473

Temel Y, Cao C, Vlamings R, Blokland A, Ozen H, Steinbusch HW, Michelsen KA, von Horsten S, Schmitz C, Visser-Vandewalle V (2006) Motor and cognitive improvement by deep brain stimulation in a transgenic rat model of Huntington's disease. Neurosci Lett 406:138–141

The Huntington's disease collaborative research group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 72:971–983

Thompson JC, Harris J, Sollom AC, Stopford CL, Howard E, Snowden JS, Craufurd D (2012) Longitudinal evaluation of neuropsychiatric symptoms in Huntington's disease. J Neuropsychiatry Clin Neurosci 24:53–60

Thompson JC, Poliakoff E, Sollom AC, Howard E, Craufurd D, Snowden JS (2010) Automaticity and attention in Huntington's disease: when two hands are not better than one. Neuropsychologia 48:171–178

Thompson JC, Snowden JS, Craufurd D, Neary D (2002) Behavior in Huntington's disease: dissociating cognition-based and mood-based changes. J Neuropsychiatry Clin Neurosci 14:37–43

Trueman RC, Brooks SP, Jones L, Dunnett SB (2007) The operant serial implicit learning task reveals early onset motor learning deficits in the Hdh knock-in mouse model of Huntington's disease. Eur J Neurosci 25:551–558

Trueman RC, Brooks SP, Jones L, Dunnett SB (2008) Time course of choice reaction time deficits in the Hdh(Q92) knock-in mouse model of Huntington's disease in the operant serial implicit learning task (SILT). Behav Brain Res 189:317–324

Trueman RC, Brooks SP, Jones L, Dunnett SB (2009) Rule learning, visuospatial function and motor performance in the Hdh(Q92) knock-in mouse model of Huntington's disease. Behav Brain Res 203:215–222

Trueman RC, Dunnett SB, Jones L, Brooks SP (2012a) Five choice serial reaction time performance in the HdhQ92 mouse model of Huntington's disease. Brain Res Bull 88:163–170

Trueman RC, Jones L, Dunnett SB, Brooks SP (2012b) Early onset deficits on the delayed alternation task in the Hdh(Q92) knock-in mouse model of Huntington's disease. Brain Res Bull 88:156–162

Twelvetrees AE, Yuen EY, Arancibia-Carcamo IL, MacAskill AF, Rostaing P, Lumb MJ, Humbert S, Triller A, Saudou F, Yan Z, Kittler JT (2010) Delivery of GABAARs to synapses is mediated by HAP1-KIF5 and disrupted by mutant huntingtin. Neuron 65:53–65

Vaccarino AL, Sills T, Anderson KE, Bachoud-Levi AC, Borowsky B, Craufurd D, Duff K, Giuliano J, Groves M, Guttman M, Kupchak P, Ho AK, Paulsen JS, Pedersen KF, Van Duijn E, Van Kammen DP, Evans K (2011) Assessment of depression, anxiety and apathy in prodromal and early Huntington disease. PLoS Curr 3: RRN1242

Van Dellen A, Cordery PM, Spires TL, Blakemore C, Hannan AJ (2008) Wheel running from a juvenile age delays onset of specific motor deficits but does not alter protein aggregate density in a mouse model of Huntington's disease. BMC Neurosci 9:34

Van Duijn E, Kingma EM, Timman R, Zitman FG, Tibben A, Roos RA, Van der Mast RC (2008) Cross-sectional study on prevalences of psychiatric disorders in mutation carriers of Huntington's disease compared with mutation-negative first-degree relatives. J Clin Psychiatry 69:1804–1810

Van Duijn E, Kingma EM, Van der Mast RC (2007) Psychopathology in verified Huntington's disease gene carriers. J Neuropsychiatry Clin Neurosci 19:441–448
Van Duijn E, Reedeker N, Giltay EJ, Roos RA, Van der Mast RC (2010) Correlates of apathy in Huntington's disease. J Neuropsychiatry Clin Neurosci 22:287–294
Van Laere K, Casteels C, Dhollander I, Goffin K, Grachev I, Bormans G, Vandenberghe W (2010) Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. J Nucl Med 51:1413–1417
Van Raamsdonk JM, Murphy Z, Slow EJ, Leavitt BR, Hayden MR (2005a) Selective degeneration and nuclear localization of mutant huntingtin in the YAC128 mouse model of Huntington disease. Hum Mol Genet 14:3823–3835
Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR (2005b) Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci 25:4169–4180
Van Wamelen DJ, Aziz NA, Anink JJ, Van Steenhoven R, Angeloni D, Fraschini F, Jockers R, Roos RA, Swaab DF (2013) Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's Disease. Sleep 36:117–125
Verny C, Allain P, Prudean A, Malinge MC, Gohier B, Scherer C, Bonneau D, Dubas F, Le Gall D (2007) Cognitive changes in asymptomatic carriers of the Huntington disease mutation gene. Eur J Neurol 14:1344–1350
Vetter JM, Jehle T, Heinemeyer J, Franz P, Behrens PF, Jackisch R, Landwehrmeyer GB, Feuerstein TJ (2003) Mice transgenic for exon 1 of Huntington's disease: properties of cholinergic and dopaminergic pre-synaptic function in the striatum. J Neurochem 85:1054–1063
Voermans NC, Petersson KM, Daudey L, Weber B, Van Spaendonck KP, Kremer HP, Fernandez G (2004) Interaction between the human hippocampus and the caudate nucleus during route recognition. Neuron 43:427–435
Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44:559–577
Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468–474
Wade A, Jacobs P, Morton AJ (2008) Atrophy and degeneration in sciatic nerve of presymptomatic mice carrying the Huntington's disease mutation. Brain Res 1188:61–68
Warby, SC., Graham, RK., and Hayden, M (2008) Huntington's disease. Gene Rev. http://www.ncbi.nim.nih.gov
Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, Duyao MP, Vrbanac V, Weaver M, Gusella JF, Joyner AL, MacDonald ME (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum Mol Genet 8:115–122
Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S, Friedlander RM, Gusella JF, Vonsattel JP, Borchelt DR, MacDonald ME (2002) Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. Hum Mol Genet 11:633–640
Wheeler VC, White JK, Gutekunst CA, Vrbanac V, Weaver M, Li XJ, Li SH, Yi H, Vonsattel JP, Gusella JF, Hersch S, Auerbach W, Joyner AL, MacDonald ME (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. Hum Mol Genet 9:503–513
White A, Dunnett SB (2006) Fronto-striatal disconnection disrupts operant delayed alternation performance in the rat. NeuroReport 17:435–441
Williams RH, Morton AJ, Burdakov D (2011) Paradoxical function of orexin/hypocretin circuits in a mouse model of Huntington's disease. Neurobiol Dis 42:438–445
Wolf RC, Gron G, Sambataro F, Vasic N, Wolf ND, Thomann PA, Saft C, Landwehrmeyer GB, Orth M (2012) Brain activation and functional connectivity in premanifest Huntington's disease during states of intrinsic and phasic alertness. Hum Brain Mapp 33:2161–2173

Wood NI, McAllister CJ, Cuesta M, Aungier J, Fraenkel E, Morton AJ (2013) Adaptation to experimental jet-lag in R6/2 mice despite circadian dysrhythmia. PLoS ONE 8:e55036

Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. J Neurosci 30:14708–14718

Yuen EY, Wei J, Zhong P, Yan Z (2012) Disrupted GABAAR trafficking and synaptic inhibition in a mouse model of Huntington's disease. Neurobiol Dis 46:497–502

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293:493–498

Zuccato C, Liber D, Ramos C, Tarditi A, Rigamonti D, Tartari M, Valenza M, Cattaneo E (2005) Progressive loss of BDNF in a mouse model of Huntington's disease and rescue by BDNF delivery. Pharmacol Res 52:133–139

Zuccato C, Marullo M, Conforti P, MacDonald ME, Tartari M, Cattaneo E (2008) Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. Brain Pathol 18:225–238

Transgenic Rat Models of Huntington's Disease

João Casaca Carreira, Ali Jahanshahi, Dagmar Zeef, Ersoy Kocabicak, Rinske Vlamings, Stephan von Hörsten and Yasin Temel

Abstract Several animal models for Huntington's disease (HD) have been created in order to investigate mechanisms of disease, and to evaluate the potency of novel therapies. Here, we describe the characteristics of the two transgenic rat models: transgenic rat model of HD (fragment model) and the Bacterial Artificial Chromosome HD model (full-length model). We discuss their genetic, behavioural, neuropathological and neurophysiological features.

Keywords Transgenic rat model · Huntington's disease · Striatum · Basal ganglia · Behaviour · BACHD · Neuropathology · Neurophysiology

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J. C. Carreira · A. Jahanshahi · D. Zeef · E. Kocabicak · R. Vlamings · Y. Temel
Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands

E. Kocabicak
Department of Neurosurgery, Ondokuz Mayis University, Samsun, Turkey

S. von Hörsten (✉)
Department of Experimental Therapy, Universitätsklinikum Erlangen, Franz-Penzoldt-Center, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany
e-mail: Stephan.V.Hoersten@ze.uni-erlangen.de

J. C. Carreira · A. Jahanshahi · D. Zeef · R. Vlamings · Y. Temel
European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands

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1 Transgenic Rat Models of HD

Huntington's disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder characterised by motor and non-motor symptoms. The mutation involves the expansion of the CAG trinucleotide repeat within exon 1 of the *HTT* gene on the short arm of chromosome 4. The result is the formation of the mutant form of the huntingtin (htt) protein. The symptoms of HD usually appear in midlife, leading to death within a period of 10–20 years. The cause of death is mainly due to complications, such as dysphagia, aspiration and overall exhaustion. Suicide is also an important cause of mortality in patients with HD. At the level of the neuropathology, late-stage HD is characterised by a profound loss of neurons, especially in the striatum and the cortex. In particular, the enkephalin positive medium spiny neurons in the striatum are substantially affected cells, already in the early stages of the disease. This leads to dysfunctional cortico-basal ganglia-thalamocortical circuits and as a result neurological and psychiatric symptoms (Frank and Jankovic 2010). For a detailed description of the symptomatology, please see other chapters in this book.

Several animal models for HD have been created in order to investigate mechanisms of disease, and to evaluate the potency of novel therapies. Here, we describe the characteristics of two transgenic rat models: transgenic model of HD (tgHD) and the Bacterial Artificial Chromosome HD (BACHD) model. Although it is more common to use mice for transgenic modifications, the advantages of having a transgenic rat model are evident. Rats have a bigger brain and skull size, allowing cranial surgical approaches more conveniently. In addition, rats can be more easily evaluated using a variety of simple and complex motor, cognitive and emotional behavioural tests. Last but not least, *Rattus norvegicus* has been the species of choice for decades in scientific environments, the pharmaceutical industry and toxicology with extensive knowledge on its behaviour and physiology generated, until only during the last few decades, when the feasibility of genetic manipulation made *Mus musculus* the mammalian species of choice in biomedical research. Despite these developments, it should not be forgotten that many pharmacological and metabolic features are closer to human responsiveness implicating a higher predictability of this species for the development of therapies in humans (Aitman et al. 2008).

1.1 Transgenic Rat Models of HD

In 1998, the first transgenic rat model of HD (tgHD) was developed by Riess and colleagues and characterised by von Hörsten and co-workers in the following years (von Horsten et al. 2003). The tgHD rats carry 51 CAG repeats under the control of the native rat *htt* promoter in the genetic background of the Sprague-Dawley outbred rat strain. The original animals were derived from a Sprague-Dawley (SD) founder oocyte (Max Delbrück Center, Berlin-Buch, Germany; MDC), by classical pronucleus microinjection using a transgene with the coding sequence of a truncated (t), mutant (m), huntingtin (htt) protein carrying 51 CAG repeats (human PCR product) under control of the endogenous rat *htt* promotor (von Horsten et al. 2003). This model is a so-called "fragment model" and more recently the tgHD rats' transgene has been transferred by selective breading onto the genetic background of Fischer 344 inbred rats (von Hörsten et al. unpublished). While SD rats (the strain of oocyte donors) represent an outbred rat strain, this transgenic line was subsequently inbred by strict brother x sister matings for ≥26 generations. According to the international nomenclature of laboratory rodents, these animals are coded "**SD/MdcSvh-Tg(tmHTT51CAG)**". Presently, for abbreviation purpose, this transgenic rat model of HD should be referred to as tgHD or "**tgHD-CAG51n**" rats, contrasting the novel BACHD rat model, recently generated.

Very recently, a second transgenic rat model has been described (Yu-Taeger et al. 2012). This BACHD transgenic rat expresses the full-length human mutant *HTT* under the control of the human *HTT* promoter and all its regulatory elements (Yu-Taeger et al. 2012).

As far as we are aware of, these models are the only published transgenic rat models of HD. A detailed description of the characteristics of these two HD models will be provided below.

2 Construction of the tgHD and BACHD Rats

The tgHD rat was developed by inserting a transgene, which was obtained from the DNA of a HD patient (19/51 CAGs) (von Horsten et al. 2003). The first 154 nucleotides of a partial huntingtin cDNA spanning 1962 base pairs (bp) of the N-terminal rat sequence (RHD10) (Schmitt et al. 1995) were replaced by a PCR (Polymerase Chain Reaction) product from the affected allele of the HD patient. The cDNA was driven by an 885 bp fragment of the rat HD promoter (position −900 to −15 bp) (Holzmann et al. 1998) and a 200 bp fragment containing the SV40 polyadenylation signal was added downstream of the cDNA resulting in RHD/Prom51A (Holzmann et al. 1998). Finally, the insert was microinjected into oocytes of Sprague-Dawley female rats (von Horsten et al. 2003). In order to verify the transgene, DNA was extracted from the tails of the offspring animals and a Southern blot was performed. Western blot analysis showed the expression

of mutant htt in the frontal and temporal cortices, hippocampus, basal ganglia and mesencephalon (Schmidt et al. 1998; von Horsten et al. 2003).

The BACHD rat was generated by microinjection of a BAC construct containing human genomic DNA expressing the full-length *HTT* gene with 97 CAG/CAA repeats, as well as all regulatory elements (Gray et al. 2008) into the oocytes of Sprague-Dawley female rats. Determination of BAC transgene integrity and genotype was done by PCR analysis of genomic DNA extracted from ear biopsy tissue (Yu-Taeger et al. 2012). The BACHD construct was designed by using a BAC containing the full 170 kb of the HTT genomic locus with approximately 20 kb upstream and 50 kb downstream flanking sequences (Yu-Taeger et al. 2012). Wild-type (WT) HTT exon 1 was replaced by mutant HTT exon 1 containing 97 mixed CAA/CAG repeats flanked by two LoxP sites. Therefore, the BAC construct permitted a conditional and inducible elimination of the mutant HTT exon 1 by Causes recombination (Cre) recombinase activity. The number of CAG repeats was analysed and the conclusion was that the polyQ encoding sequence was stable in different brain regions, at different ages and gender over the generations (Yu-Taeger et al. 2012).

2.1 Behavioural Phenotype

In this section we will describe the motor and non-motor features of both rat models of HD. The tgHD rats have been characterised reasonably well. However, the BACHD model has recently become available for studies, and therefore to this end there are only limited data available.

2.2 Motor Symptoms

The motor symptoms of the tgHD rats have been reasonably well studied. Gait and balance abnormalities, hyper and hypokinetic features and chorea-like movements are the main motor symptoms observed in this model (Zeef et al. 2012b; von Horsten et al. 2003; Cao et al. 2006; Hohn et al. 2011; Nguyen et al. 2006; Vandeputte et al. 2010; Ortiz et al. 2012). The accelerod is a test that is used to assess motor coordination and balance of the fore and hind limbs (von Horsten et al. 2003). While at younger ages being significantly better than corresponding WT littermate controls, at 6 months of age, tgHD rats show a significant decreased performance when compared to WT animals (Nguyen et al. 2006). The performance on the accelerod worsens at 10 months of age, indicating a progression of the symptoms (von Horsten et al. 2003). Using a different test for gait and balance, the force-plate actometer, abnormalities were found in 12–15-month-old animals (Ortiz et al. 2012).

Another characteristic motor abnormality is the hypermobility in these animals, already at the early stage of the disease. In the open field test, a test to assess spontaneous locomotion and generalised anxiety-like ("emotional") behaviour, the animals show increased mobility times (Zeef et al. 2012b). This hypermobility decreases after the age of 12 months, and is probably indicative of bradykinesia at the later stage.

An interesting movement disorder, again observed in the fragment tgHD model, is the chorea-like movement. These are abrupt, rapid, brief and unsustained irregular movements of the neck, also classified as opisthotonus-like movements (Cao et al. 2006; von Horsten et al. 2003). These movements, which are only seen at the level of the neck, have similarities with the human HD chorea (Cao et al. 2006). It is also claimed that the tgHD rat model is the first animal model to show this type of choreiform movement disorder (Cao et al. 2006). The frequency of the choreiform movements increases with disease progression and are present till death in these animals.

The BACHD animals have been evaluated using the rotarod test and the footprint analysis method (Yu-Taeger et al. 2012). The rotarod performance was significantly worse in the transgenic animals already at 1 month of age, which further deteriorated with disease progression. Older animals, 14 months of age, showed also gait abnormalities in the footprint analysis. They made shorter steps (Yu-Taeger et al. 2012).

2.3 Non-motor Symptoms

The non-motor symptoms are an important aspect of the disease, since patients with HD often are suffering more from the non-motor symptoms when the disease progresses.

The tgHD rats show several non-motor deficits including cognitive and emotional disturbances at the early and late stages of the disease. The onset of these symptoms is usually prior to the development of the classical motor symptoms. Several studies have investigated the non-motor features of the tgHD rats (Kirch et al. 2013; Lawrence et al. 1996; Vlamings et al. 2012b; Nguyen et al. 2006; Zeef et al. 2012a, b; Cao et al. 2006). Cognitive decline starts between 6 and 9 months of age and this decline increases with ageing (Nguyen et al. 2006). Animals have difficulty with acquisition in the water maze and the double-H maze tasks (Kirch et al. 2013). In the object location task and object recognition task, tgHD rats show impaired visuospatial and visual object memory (Zeef et al. 2012a). Reduced spatial learning and working memory has been reported as well (Ortiz et al. 2012).

Impulsivity-related symptoms have been observed in the tgHD rats. Increased numbers of premature responses were found in the choice reaction time test, which increased with disease progression (Cao et al. 2006). Another non-motor feature of the tgHD rats is the impaired anxiety levels. Reduced anxiety-like behaviour was

noticed in the social interaction test when compared to the WT littermates (Nguyen et al. 2006). Similar findings were found in the open field test and the elevated zero maze, already at a very early stage of the disease (Zeef et al. 2012b).

The BACHD rats have been evaluated using an automated homecage tracking system. The animals showed decreased exploratory behaviour and similarly to the tgHD rats, decreased levels of anxiety-related behaviour (Yu-Taeger et al. 2012).

2.4 Neuropathological Phenotype

From a neuropathological perspective, in the tgHD rats intranuclear polyglutamine (polyQ) aggregates and neuropil aggregates can be found (Nguyen et al. 2006). PolyQ aggregates have been detected in the caudate-putamen, thalamus, substantia nigra pars compacta and the deep layers of the cortex (Nguyen et al. 2006). There is a profound progressive striatal cell loss in tgHD rats, contributing to reduced striatal volumes and enlarged ventricles (Kantor et al. 2006). In addition, we observed at the later stages of the disease, a profound cortical thinning (Fig. 1). Another neuropathological finding in this animal model was the significantly higher number of dopaminergic cells in the substantia nigra pars compacta, ventral tegmental area and the dorsal raphe nucleus, leading to an enhanced dopamine release into the dorsal and ventral striatum (Jahanshahi et al. 2010; Jahanshahi et al. 2013). This hyperdopaminergic status has been linked to the choreiform movements observed in this model (Fig. 2).

Neuropathologically, BACHD rats develop polyQ aggregates in axons, synaptic terminals, as well as dark neurons (Yu-Taeger et al. 2012). There are also early changes in the pattern of the striosome and matrix compartments, accompanied by a decrease in the total and mean striosome area (Yu-Taeger et al. 2012).

2.5 Neurophysiological Phenotype

The metabolic and neuronal activities of basal ganglia nuclei in the tgHD rats have been investigated at different levels. First, the overall neuronal activity at a supracellular level, by cytochrome oxidase histochemistry was determined. Second, the subcellular metabolic activity was assessed, by immunohistochemistry for peroxisome proliferator-activated receptor-γ transcription co-activator (PGC-1α), a key player in the mitochondrial machinery. Finally, extracellular single unit recordings were performed to determine the cellular activity. Results showed a significantly increased cytochrome oxidase levels in the globus pallidus and subthalamic nucleus in the tgHD animals. PGC-1α expression was only enhanced in the subthalamic nucleus and electrophysiological recordings revealed decreased firing frequency of the majority of the neurons in the globus pallidus and increased

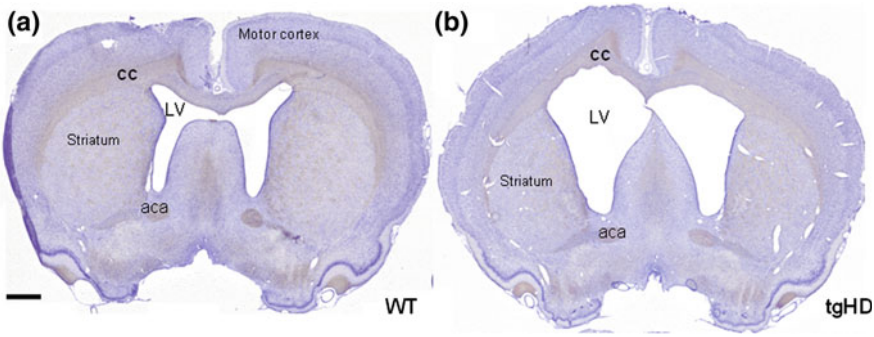

Fig. 1 Representative low-power photomicrographs, showing Nissl stained coronal brain sections of a 16-month-old WT and a 16-month-old transgenic Huntington's disease rat (tgHD). Note the striatal volume and cortical volume loss in the tgHD rat. There is an evident enlargement of the lateral ventricles, which is considered as a *ex vacuo* dilatation effect due to tissue loss. *cc* corpus callosum, *LV* lateral ventricle, *aca* anterior commissure ant, scale bar = 1 mm

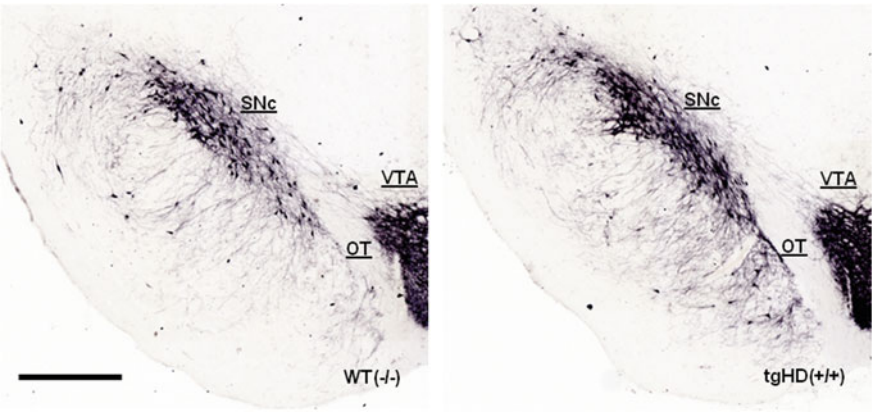

Fig. 2 Representative low-power photomicrographs of frontal brain sections stained for *TH* tyrosine hydroxylase showing the *SNc* substantia nigra pars compacta, *OT* optic tract and a small part of the *VTA* ventral tegmental area of a WT littermate rat and a transgenic HD homozygous (+/+) rat. Note the increased TH containing cell density in the SNc of the transgenic HD rat upon close inspection adopted from Jahanshahi et al. 2010. Scale bar is approximately 500 μm

firing frequency of the majority of the neurons in the subthalamic nucleus. These data suggest that the globus pallidus and subthalamic nucleus play a role in the neurobiology of HD (Vlamings et al. 2012a). Another line of data suggested already an impaired corticostriatal information processing using a combined electrophysiological-behavioural approach (Hohn et al. 2011).

The BACHD rats are being characterised in terms of neurophysiological activity of brain structures.

3 Conclusion

To our knowledge there are two published transgenic rat models of HD. The first is the tgHD rat model and the second is the BACHD model. The tgHD model is a so-called "fragment model" and the BACHD is a full-length model. The tgHD rats are relatively well characterised (Table 1).

The tgHD rats have a slow progressive behavioural and neuropathological phenotype. In general, the symptoms can be divided into two stages: early and late. The early stage is characterised by hypermobility and reduced anxiety behaviour. Although some scientists have found early subtle cognitive changes, in our hands these do not have a large impact on the animals. This early stage is not accompanied with striatal cell degeneration or cortical cell damage, which occur later in the disease (Kantor et al. 2006; Nguyen et al. 2006). Nevertheless, there might already be striatal cell dysfunction (Miller et al. 2010). Already some choreiform movements can be seen in this early stage. We think that this stage ends around 11–12 months of age. The later stage is mainly characterised by the presence of more choreiform movements. Upon testing, the animals show impaired cognitive functioning and features of increased impulsivity (Cao et al. 2006; Zeef et al. 2012a). There is a profound loss of striatal cells and signs of cortical cell damage (Kantor et al. 2006). The life expectancy of these animals is shorter than controls, probably a few months (unpublished observations).

There are a few issues that need to be considered in this animal model. The first is the effect of sex. There are differences in the behavioural phenotype between the sexes (Bode et al. 2008). This needs to be taken into account when comparing sets of data. In our studies, we often used males. The second is the gene-dose effect. We have been working with both homozygous and hemizygous animals. Homozygous animals show more robust behavioural and neuropathological features (Cao et al. 2006; Kantor et al. 2006), mimicking human HD. We decided to work only with homozygous rats. The third issue is a potential gene-drift effect. In a recent publication, researchers could not establish robust cognitive changes in this animal model (Fielding et al. 2012; Brooks et al. 2009). One explanation could be a potential gene-drift, but the existence of such mechanism still needs to be demonstrated. Furthermore, standardization of breeding might be an issue of differences observed between labs. TgHD rats should be bred by strict brother x sister matings of hemizygous (HET) males and females. From the offspring of 25 % WT, 50 % HET, and 25 % HOM animals, in most studies male WT littermates were compared with male HOM transgenics. In our colonies of animals, we have observed slight differences between generations of further inbred homozygous rats, but consistently found the clear behavioural and neuropathological phenotypes as described above. The congenic tgHD rat line on F344 rat genetic background confirms the impact of the truncated mHtt transgene in producing an HD-like phenotype in rats, even in front of a different genetic

Table 1 This table summarises the main findings with respect to the different features of the tgHD rats

Study authors	Domain	Test/Procedure	Main findings	Age
	Cognition			
Nguyen et al. (2006)		Radial maze	Impaired working memory	9 and 12 months
Zeef et al. (2012b)		Object recognition test and object location test	Deficits in visuospatial and visual object memory at early and late stages in tgHD rats 120	10 and 16 months
Cao et al. (2006)		Choice reaction time task	Decreased number of correct responses and increased response bias in tgHD rats	15–20 months
Kirch et al. (2013)		Water-maze task and Double-H maze	Deficit in acquisition, poorer retention, more procedural errors and the learning process is slower in tgHD rats compared with WT and heterozygous animals	6 and 12 months
Kántor et al. (2006)		Choice reaction time task	tgHD rats showed increased premature responses, reduced number of correct responses and higher response bias	14 months
	Emotion			
Nguyen et al. (2006)		Social interaction test	HD animals showed reduced anxiety-like behaviour	1 month
Von Hörsten et al. (2003), Nguyen et al. (2006)		Elevated plus maze test	tgHD animals presented decreased anxiety-like behaviour	3 months
Zeef et al. (2012a)		Elevated zero maze	Reduced anxiety-like behaviour in tgHD	7, 8 and 10 months
Zeef et al. (2012a)		Open field test	Reduced anxiety in tgHD animals compared with WT	6 and 7 months
	Motor			
Nguyen et al. (2006)		Accelerod test	Bimodal; initial improvements are followed in tgHD rats' reduced balance and motor coordination	2 months; progressive worsening from 6 months onwards
Nguyen et al. (2006)		Beam walking test	tgHD showed impaired fine motor coordination and balance	9 months
Zeef et al. (2012a, b)		Open field test	Hyperkinetic feature in tgHD	6, 7, 8 and 10 months
Cao et al. (2006)		Choreiform movements	Significant increased number of choreiform movements in homozygous tgHD animals	20 months
Ortiz et al. (2012)		Gait analysis	Gait disturbances	12–15 months

(continued)

Table 1 (continued)

Study authors	Domain	Test/Procedure	Main findings	Age
	Pathology			
Von Hörsten et al. (2003), Nguyen et al. (2006)		Aggregates	Aggregation foci in thalamus, substantia nigra pars compacta, cortex and caudate-putamen	9 months
Von Hörsten et al. (2003), Nguyen et al. (2006)		Striatal atrophy	Decreased striatal volume in tgHD rats	12 months
Jahanshahi et al. (2013)		Brain stem analysis	Increased number of dopamine cells and decreased number of serotonin-containing cells in Dorsal Raphe Nucleus in tgHD	11 months
Vlamings et al. (2012a, b)		Electrophysiological changes in basal ganglia	Increased cytochrome oxidase levels in globus pallidus and subthalamic nucleus of tgHD PGC-1alfa increased in the subthalamic nucleus Decreased firing frequency in globus pallidus and increased firing frequency in the subthalamic nucleus	10–12 months
Verwaest et al. (2011)		Metabolic changes in tgHD rats	N-acetylaspartate decreased in serum. Increased glutamine, succicinic acid, glucose and lactate in serum. Lactate and glucose increased in CSF	2 months
Ortiz et al. (2012)		Dopamine release measurements	Decreased dopamine release in tgHD rats after application of single and multiple electrical stimulus at different frequencies	20–26 months
Hohn et al. (2011)		Prefontostriatal function	In tgHD rats, poorer temporal sensitivity was found in a bisection task and assessment of field- potentials showed enhanced plasticity at prefrontostriatal afferents	4 months
Kantór et al. (2006)		Stereological analysis	Reduction of striatal volume (more pronounced in the medial paraventricular striatum) in tgHD animals	12 months
Jahanshahi et al. (2010)		Stereological analysis of ventral tegmental area and substantia nigra pars compacta	Significantly increased tyrosine-hydroxylase immunoreactive cells in the VTA and SNc when compared with controls. Higher TH expression in dorsal and ventral striatum as compared with controls	11 months
Nguyen et al. (2006)		Striatal atrophy	Decreased striatal volume in tgHD rats	12 months
Jahanshahi et al. (2013)		Brain stem analysis	Increased number of dopamine cells and decreased number of serotonin-containing cells in the dorsal raphe nucleus	11 months
	Treatment			
Temel et al. (2004)		Deep brain stimulation	Globus pallidus stimulation improved cognitive dysfunction and reduction of chorea movements	20 months

Details regarding the first author, year of publication and the age of the animals are provided as well

Table 2 This table summarises the main findings with respect to the different features of the BACHD rats

Study authors	Domain	Test/Procedure	Main findings	Age
Yu-Taeger et al. (2012)	Emotion			
		Elevated plus maze	Reduced anxiety-like behaviour	1, 4 and 12 months
	Motor			
		Accelerated rotarod	Balance problems with subsequent falls	Progressive worsening from 1 month onwards till 15 months
		Footprint analysis	Shorter steps and reduced overlap limbs	14 months
		Automated home cage-like environment	Reduced rearing and locomotor activity in BACHD animals	Only at 3- and 6-months old
	Pathology			
		Htt aggregates	In the cortex, nucleus accumbens, bed nucleus of stria terminalis and hippocampus.	3 months
		Electron microscopy	*Htt* deposits are found in axons and synaptic terminals	13 months
		Dynamic PET scan in the striatum	Reduction of dopamine receptor binding	18 months 6 months
	Metabolic			
		Food intake	Reduced food consumption	3–18 months

These findings were reported by Yu-Taeger et al. 2012

background. After evaluating the different phenotypes of the tgHD rats, we consider the tgHD rat model suitable to evaluate therapeutic approaches.

The phenotype of the BACHD model in terms of behaviour and neuropathology seems to be clinically relevant and therefore it is a promising model (Table 2). However, thus far there are no hyperkinetic movements observed in these animals. The model needs more characterisation to draw final conclusions.

There are differences between the two models in terms of construct validity as well, such as the number of trinucleotide repeats (51 CAG in tgHD vs. 97 CAG-CAA in BACHD), being a fragment model (tgHD) versus a full-length model (BACHD), using homozygous animals (tgHD) versus heterozygous (BACHD), keeping an inbred background (tgHD) versus outbred background (BACHD), which should be taken into account when a model is chosen for a specific purpose.

References

Aitman TJ, Critser JK, Cuppen E, Dominiczak A, Fernandez-suarez XM, Flint J, Gauguier D, Geurts AM, Gould M, Harris PC, Holmdahl R, Hubner N, Izsvak Z, Jacob HJ, Kuramoto T, Kwitek AE, Marrone A, Mashimo T, Moreno C, Mullins J, Mullins L, Olsson T, Pravenec M, Riley L, Saar K, Serikawa T, Shull JD, Szpirer C, Twigger SN, Voigt B, Worley K (2008) Progress and prospects in rat genetics: a community view. Nat Genet 40:516–522

Bode FJ, Stephan M, Suhling H, Pabst R, Straub RH, Raber KA, Bonin M, Nguyen HP, Riess O, Bauer A, Sjoberg C, Petersen A, Von horsten S (2008) Sex differences in a transgenic rat model of Huntington's disease: decreased 17beta-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. Hum Mol Genet 17: 2595–2609

Brooks S, Fielding S, Dobrossy M, Von horsten S, Dunnett S (2009) Subtle but progressive cognitive deficits in the female tgHD hemizygote rat as demonstrated by operant SILT performance. Brain Res Bull 79:310–315

Cao C, Temel Y, Blokland A, Ozen H, Steinbusch HW, Vlamings R, Nguyen HP, Von horsten S, Schmitz C, Visser-vandewalle V (2006) Progressive deterioration of reaction time performance and choreiform symptoms in a new Huntington's disease transgenic ratmodel. Behav Brain Res 170:257–261

Fielding SA, Brooks SP, Klein A, Bayram-weston Z, Jones L, Dunnett SB (2012) Profiles of motor and cognitive impairment in the transgenic rat model of Huntington's disease. Brain Res Bull 88:223–236

Frank S, Jankovic J (2010) Advances in the pharmacological management of Huntington's disease. Drugs 70:561–571

Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195

Hohn S, Dallerac G, Faure A, Urbach YK, Nguyen HP, Riess O, Von horsten S, Le blanc P, Desvignes N, El massioui N, Brown BL, Doyere V (2011) Behavioral and in vivo electrophysiological evidence for presymptomatic alteration of prefrontostriatal processing in the transgenic rat model for huntington disease. J Neurosci 31:8986–8997

Holzmann C, Maueler W, Petersohn D, Schmidt T, Thiel G, Epplen JT, Riess O (1998) Isolation and characterization of the rat huntingtin promoter. Biochem J 336(Pt 1):227–234

Jahanshahi A, Vlamings R, Kaya AH, Lim LW, Janssen ML, Tan S, Visser-vandewalle V, Steinbusch HW, Temel Y (2010) Hyperdopaminergic status in experimental Huntington disease. J Neuropathol Exp Neurol 69:910–917

Jahanshahi A, Vlamings R, Roon-mom WV, Faull RL, Waldvogel HJ, Janssen ML, Yakkioui Y, Zeef D, Kocabicak E, Steinbusch HW, Temel Y (2013). Changes in brainstem serotonergic and dopaminergic cell populations in experimental and clinical Huntington's disease. Neuroscience 238:71–81

Kantor O, Temel Y, Holzmann C, Raber K, Nguyen HP, Cao C, Turkoglu HO, Rutten BP, Visser-vandewalle V, Steinbusch HW, Blokland A, Korr H, Riess O, Von horsten S, Schmitz C (2006) Selective striatal neuron loss and alterations in behavior correlate with impaired striatal function in Huntington's disease transgenic rats. Neurobiol Dis 22:538–547

Kirch RD, Meyer PT, Geisler S, Braun F, Gehrig S, Langen KJ, Von horsten S, Nikkhah G, Cassel JC, Dobrossy MD (2013) Early deficits in declarative and procedural memory dependent behavioral function in a transgenic rat model of Huntington's disease. Behav Brain Res 239:15–26

Lawrence AD, Sahakian BJ, Hodges JR, Rosser AE, Lange KW, Robbins TW (1996) Executive and mnemonic functions in early Huntington's disease. Brain 119(Pt 5):1633–1645

Miller BR, Walker AG, Fowler SC, Von horsten S, Riess O, Johnson MA, Rebec GV (2010) Dysregulation of coordinated neuronal firing patterns in striatum of freely behaving transgenic rats that model Huntington's disease. Neurobiol Dis 37:106–113

Nguyen HP, Kobbe P, Rahne H, Worpel T, Jager B, Stephan M, Pabst R, Holzmann C, Riess O, Korr H, Kantor O, Petrasch-parwez E, Wetzel R, Osmand A, Von horsten S (2006) Behavioral abnormalities precede neuropathological markers in rats transgenic for Huntington's disease. Hum Mol Genet 15:3177–3194

Ortiz AN, Osterhaus GL, Lauderdale K, Mahoney L, Fowler SC, Von horsten S, Riess O, Johnson MA (2012) Motor function and dopamine release measurements in transgenic Huntington's disease model rats. Brain Res 1450:148–156

Schmidt T, Landwehrmeyer GB, Schmitt I, Trottier Y, Auburger G, Laccone F, Klockgether T, Volpel M, Epplen JT, Schols L, Riess O (1998) An isoform of ataxin-3 accumulates in the nucleus of neuronal cells in affected brain regions of SCA3 patients. Brain Pathol 8:669–679

Schmitt I, Bachner D, Megow D, Henklein P, Hameister H, Epplen JT, Riess O (1995) Expression of the Huntington disease gene in rodents: cloning the rat homologue and evidence for downregulation in non-neuronal tissues during development. Hum Mol Genet 4:1173–1182

Temel Y, Visser-Vandewalle V, van der Wolf M, Desbonnet L, Spincemaille GH, Hoogland G, Steinbusch HWM (2004) Monopolar versus bipolar high frequency stimulation of the rat subthalamic nucleus: differences in histological damage. Neurosci Lett 367(1):92–96

Vandeputte C, Taymans JM, Casteels C, Coun F, Ni Y, Van laere K, Baekelandt V (2010) Automated quantitative gait analysis in animal models of movement disorders. *BMC* Neurosci 11:92

Verwaest KA, Vu TN, Laukens K, Clemens LE, Nguyen HP, Van Gasse B, Martins JC, Van der Linden A, Dommisse R (2011) (1)H NMR based metabolomics of CSF and blood serum: a metabolic profile for a transgenic rat model of Huntington's disease. Biochim Biophys Acta Nov;1812(11):1371–1379. doi:10.1016/j.bbadis.2011.08.001

Vlamings R, Benazzouz A, Chetrit J, Janssen ML, Kozan R, Visser-vandewalle V, Steinbusch HW, Von horsten S, Temel Y (2012a) Metabolic and electrophysiological changes in the basal ganglia of transgenic Huntington's disease rats. Neurobiol Dis 48:488–494

Vlamings R, Zeef DH, Janssen ML, Oosterloo M, Schaper F, Jahanshahi A, Temel Y (2012b) Lessons learned from the transgenic Huntington's disease rats. Neural Plast 2012:682712

Von horsten S, Schmitt I, Nguyen HP, Holzmann C, Schmidt T, Walther T, Bader M, Pabst R, Kobbe P, Krotova J, Stiller D, Kask A, Vaarmann A, Rathke-hartlieb S, Schulz JB, Grasshoff U, Bauer I, Vieira-saecker AM, Paul M, Jones L, Lindenberg KS, Landwehrmeyer B, Bauer A, Li XJ, Riess O (2003) Transgenic rat model of Huntington's disease. Hum Mol Genet 12:617–624

Yu-taeger L, Petrasch-parwez E, Osmand AP, Redensek A, Metzger S, Clemens LE, Park L, Howland D, Calaminus C, Gu X, Pichler B, Yang XW, Riess O, Nguyen HP (2012) A novel BACHD transgenic rat exhibits characteristic neuropathological features of Huntington disease. J Neurosci 32:15426–15438

Zeef DH, Van goethem NP, Vlamings R, Schaper F, Jahanshahi A, Hescham S, Von horsten S, Prickaerts J,Temel Y (2012a) Memory deficits in the transgenic rat model of Huntington's disease. Behav Brain Res 227:194–198

Zeef DH, Vlamings R, Lim LW, Tan S, Janssen ML, Jahanshahi A, Hoogland G, Prickaerts J, Steinbusch HW, Temel Y (2012b) Motor and non-motor behaviour in experimental Huntington's disease. Behav Brain Res 226:435–439

Large Animal Models of Huntington's Disease

Xiao-Jiang Li and Shihua Li

Abstract Huntington's disease is caused by the expansion of a polyglutamine repeat (> 37 glutamines) in the disease protein huntingtin, which results in preferential neuronal loss in distinct brain regions. Mutant huntingtin causes late-onset neurological symptoms in patients in middle life, though the expression of mutant huntingtin is ubiquitous from early life. Thus, it is important to understand why mutant huntingtin selectively causes neuronal loss in an age-dependent manner. Transgenic animal models have been essential tools for uncovering the pathogenesis and therapeutic targets of neurodegenerative diseases. Genetic mouse models have been investigated extensively and have revealed the common pathological hallmark of age-dependent formation of aggregates or inclusions consisting of misfolded proteins. However, most genetic mouse models lack striking neurodegeneration that has been found in patient brains. Since there are considerable species differences between small and large animals, large animal models of Huntington's disease may allow one to identify the pathological features that are more similar to those in patients and also help uncover more effective therapeutic targets. This chapter will focus on the important findings from large animal models of Huntington's disease and discusses the use of large animal models to investigate the pathogenesis of Huntington's disease and develop new therapeutic strategies.

Keywords Aging · Huntington's disease · Neurodegeneration · Species differences · Polyglutamine · Transgenic animals

X.-J. Li (✉) · S. Li
Department of Human Genetics, Emory University School of Medicine, 615 Michael St 347, Atlanta, GA 30322, USA
e-mail: xli2@emory.edu

X.-J. Li
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No.1 West Beichen Road, Chaoyang District 100101 Beijing, China

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1 Introduction

Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and several other neurodegenerative diseases share a common pathological feature, which is selective neurodegeneration that occurs in an age-dependent manner. All these diseases are caused by misfolded proteins that form aggregates in the brains of affected patients. For example, in the brains of patients with AD, extracellular aggregates (senile plaques), which are formed by beta amyloid (Aβ) proteins, are the pathological hallmark of AD, whereas Lewy bodies, which contain alpha-synuclein, are a characteristic brain pathology of patients with PD. In HD, nuclear inclusions and neuropil aggregates are formed by N-terminal mutant huntingtin fragments that carry more than 37 polyglutamine repeats (Gusella et al. 1993; Vonsattel and DiFiglia 1998). Although whether these aggregates are toxic or protective remains to be investigated, it is clear that these aggregates are formed by small misfolded peptides and result from the accumulation of misfolded proteins. Consistently, the age-dependent formation of these aggregates in patient brains is correlated with the progression of neurological symptoms in AD, PD, and HD.

All these neurodegenerative diseases affect distinct types of neuronal cells despite the widespread expression of the disease proteins. For example, AD mainly affects the cortical neurons, whereas PD selectively affects dopaminergic neurons in the brain region known as the substantia nigra. HD, on the other hand, preferentially affects the medium spiny neurons in the striatum (Vonsattel et al. 1998). The selective neuropathology in each disease indicates that protein context is important for the specific neuronal toxicity in these diseases. Because all these disease proteins have different functions and interact with different partners, protein misfolding is likely to alter their functions and association with other proteins, resulting in a gain of toxicity in specific types of neurons.

HD shares common pathological changes with other neurodegenerative diseases but is caused by an autosomal dominant genetic mutation, making HD an ideal model to study how protein misfolding leads to selective neurodegeneration. The mechanistic insight obtained from studying HD would be very helpful for

understanding other neurodegenerative diseases, such as AD and PD, which are also caused by protein misfolding. Thus, this chapter will focus on HD and its animal models.

2 HD and Polyglutamine Expansion

HD is characterized by motor dysfunction, cognitive decline, and psychological dysfunction. HD displays selective neurodegeneration that occurs preferentially in the brain striatum (Gusella et al. 1993; Vonsattel and DiFiglia 1998). The majority of patients with HD show symptoms in midlife and often die 10–15 years after the onset of symptoms. The genetic cause of HD is the expansion of a CAG repeat (>36 CAGs) in exon1 of the HD gene. Thus, the CAG repeat expansion results in an expanded polyglutamine (polyQ) tract in the N-terminal region of htt, a large-sized protein (3,144 amino acids) that is ubiquitously expressed in various types of cells and interacts with a number of proteins (Harjes and Wanker 2003; Li and Li 2004). As a result, mutant htt with an expanded polyQ tract forms insoluble aggregates or inclusions in the brains of patients with HD in an age-dependent manner.

The selective neurodegeneration in HD occurs early in the medium spiny neurons in the striatum. Other brain regions, such as the deep layers of the cortex, the hypothalamus, and the hippocampus, also undergo neurodegeneration in the later stages of HD (Vonsattel et al. 1998). While the primary function of htt has yet to be determined, it is known to be essential for early development and probably plays a role in cellular trafficking as a scaffold protein (Harjes and Wanker 2003; Li and Li 2004). It is evident that only N-terminal mutant htt is able to form aggregates and is more toxic than full-length mutant htt (Gutekunst et al. 1999; Zhou et al. 2003), which has led to extensive studies to identify the proteolytic cleavage sites mediated by various proteases, including calpains, aspartyl proteases, and caspases, which can degrade mutant htt to generate N-terminal htt fragments (Qin and Gu 2004). In addition to having the abilities to misfold and aggregate, N-terminal mutant htt fragments can also accumulate in the nucleus, whereas the majority of full-length mutant htt remains in the cytoplasm (DiFiglia et al. 1997; Gutekunst et al. 1999). The nuclear localization of N-terminal mutant htt can lead to abnormal binding of mutant htt to various transcription factors, subsequently affecting transcriptional expression (Harjes and Wanker 2003; Li and Li 2004).

3 HD Mouse Models

Identification of the genetic mutation for HD has led to the establishment of various transgenic HD mouse models. These models include transgenic mice (R6/2, N171-82Q) expressing N-terminal mutant htt (Davies et al. 1997; Schilling et al.

1999), full-length mutant htt transgenic mice (YAC and BAC) (Slow et al. 2003; Gray et al. 2008), and HD repeat knock-in (KI) mice (Wheeler et al. 2000; Lin et al. 2001; Menalled et al. 2002). R6/2 and N171-82Q mice display abundant htt aggregates in their brains at 3–4 months, as well as severe neurological symptoms and earlier death at 3–6 months (Davies et al. 1997; Schilling et al. 1999). Yeast artificial chromosome transgenic mice (YAC128), HD KI mice, and BACHD transgenic mice, which express full-length mutant human htt with an expanded polyQ repeat (114–150Q), display obvious htt aggregates only at older ages (7–10 months), show milder neurological symptoms than R/2 and N171-82Q mice, and can survive as wild-type mice (Lin et al. 2001; Slow et al. 2003; Gray et al. 2008; Wang et al. 2008). A careful analysis of HD150Q KI mice at 22 months, however, demonstrated that these mice develop the well-characterized htt aggregates, which could be seen in R6/2 mice at the age of 12 weeks (Woodman et al. 2007). Thus, characterization of various HD mouse models provides clear evidence that small N-terminal htt fragments with expanded polyQ tracts become misfolded and form aggregates. Consistently, polyQ-containing N-terminal htt fragments also form aggregates in HD cellular models (Li and Li 1998) and in HD patient brains (DiFiglia et al. 1997; Gutekunst et al. 1999). Despite the milder phenotypes of HD mice that express full-length mutant htt, such as YAC128 and HD knock-in mice, these mice show preferential accumulation of mutant htt in the striatum, consistent with the preferential loss of the medium spiny neurons in the striatum of HD patients. Thus, the accumulation of mutant htt in neuronal cells is clearly a prerequisite for neuronal dysfunction and degeneration.

Since mutant htt is ubiquitously expressed in various types of cells and localized in different subcellular regions including the nucleus and synapses, it would be important to investigate how mutant htt in different cell types and subcellular localization contribute to the disease progression. Transgenic mouse models allow for the selective expression mutant htt in neuronal and glial cells in the brain. Expression of N-terminal mutant htt in astrocytes is sufficient to cause age-dependent neurological symptoms (Bradford et al. 2009). Recently, transgenic mice that selectively express N-terminal mutant htt in presynaptic terminals were established and also show severe neurological phenotypes, providing convincing evidence for the critical role of synaptic mutant htt in the disease (Xu et al. 2013).

Although HD mouse models have been used widely to uncover the pathogenesis of HD and to develop treatments, most of these mouse models show no apoptosis or overt neurodegeneration in their brains. Similarly, in other polyQ mouse models, the lack of striking neurodegeneration is also a noteworthy phenomenon. Further, transgenic mice for AD and PD do not show typical neurodegeneration, either (Lee et al. 2012; LaFerla and Green 2012), although neurodegeneration is the major pathological event in AD and PD patients (Mattson 2000; Yuan and Yankner 2000). All these facts point out that, although mouse models are used widely to investigate the pathogenesis of HD and other neurological diseases, they have their limitations and do not replicate the full range of neurological phenotypes seen in human diseases. Such limitations reflect the importance of species differences in the development of neurodegeneration.

4 The Large Animal Models of HD

Although biological differences between humans and mice may account for the failure of some mouse models to replicate pathology in humans, whether larger transgenic animal models can mimic important neurodegenerative features caused by misfolded proteins remains to be rigorously tested. The creation of a transgenic monkey in 2001 (Chan et al. 2001) demonstrated that the monkey genome could be genetically modified and has led to the generation of transgenic nonhuman primate models expressing disease genes or exogenous foreign genes (Yang et al. 2008; Sasaki et al. 2009; Niu et al. 2010). Of which, transgenic HD rhesus monkeys express exon1 mutant htt with 84Q under the control of the human ubiquitin promoter (Yang et al. 2008). These HD monkeys were generated by injecting lentiviruses into fertilized oocytes to express mutant htt. Unlike transgenic mice, which can survive after birth when expressing the same exon1 mutant htt with an even longer polyQ repeat (150Q) (Davies et al. 1997; Cheng et al. 2013), HD transgenic monkeys with 84Q could die postnatally and this early death is associated with the levels of mutant htt (Yang et al. 2008). Despite their early death, some transgenic monkeys developed key clinical HD features including dystonia, chorea, and seizure (Yang et al. 2008), which have not been replicated by mouse models and other small animal models. Like the brains of HD mouse models and patients, the HD monkey brains also show abundant htt aggregates in the neuronal nuclei and neuronal processes (Fig. 1a). More importantly, the transgenic HD monkeys display the degeneration of axons and neuronal processes in the absence of obvious cell body degeneration, suggesting that neuronal degeneration in HD may initiate from neuronal processes (Fig. 1b). Such findings provide us with valuable information to understand the pathogenesis of HD.

Our collaboration with Dr. Liangxue Lai at the Guangzhou Institutes of Biomedicine and Health (GIBH), Chinese Academy of Sciences, also led to the generation of transgenic HD pigs that express N-terminal mutant htt consisting of the first 208 amino acids with 105Q (N208-105Q) (Yang et al. 2010). The transgenes were expressed under the control of the cytomegalovirus enhancer and chicken beta-actin (CAG) promoter to allow the ubiquitous expression of transgenes in all tissues. Primary porcine fetal fibroblast cells expressing this mutant htt fragment were used to generate transgenic HD pigs via nuclear transfer. Six early pregnancies were established, and four of them went to term, with five live births. Like transgenic monkey models of HD, most of these transgenic HD piglets die postnatally, and some transgenic HD pigs show a severe chorea phenotype before death. We also generated transgenic mice expressing the same mutant htt and found that transgenic HD mice could live up to 9 months. Thus, the postnatal death of transgenic HD piglets also suggests that mutant htt is more toxic to larger animals. More importantly, in all transgenic pig brains examined, there were apoptotic cells (Fig. 2, Yang et al. 2010), which have not been reported in any HD mouse models.

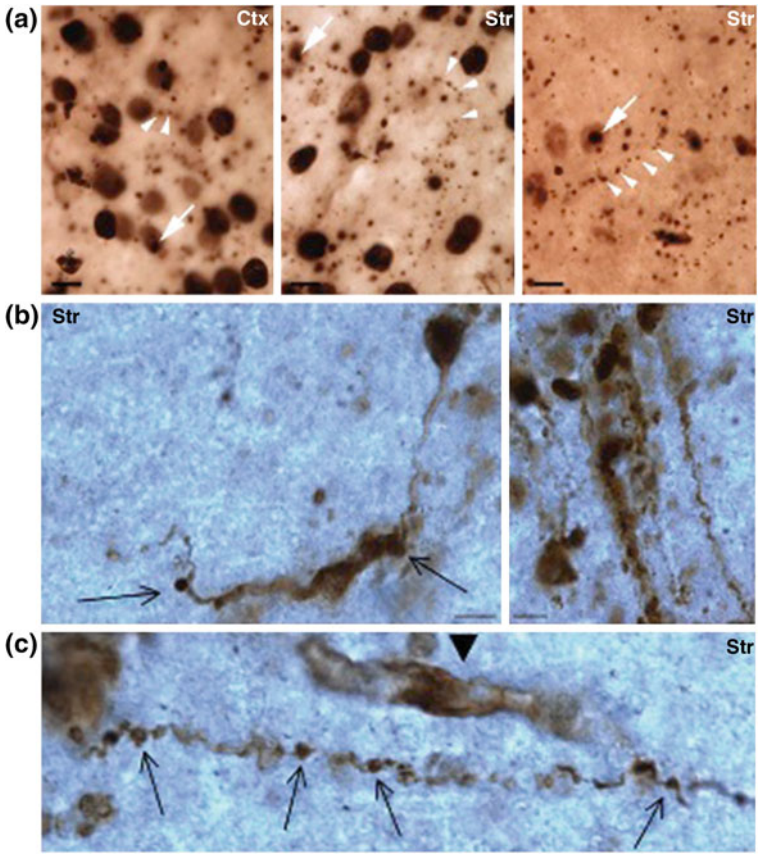

Fig. 1 Axonal degeneration in transgenic HD monkey brains. (**a**) Htt (EM48) immunostaining showing the presence of htt aggregates (*arrows*) in the neuronal nuclei in the cortex and striatum and in the neuronal processes (multiple arrowheads). (**b**) Htt immunostaining also revealed axonal degeneration (*arrows*) in HD monkey brain. *Arrowhead* indicates a glial cell

However, transgenic HD sheep expressing full-length mutant htt with a 73Q tract live normally and show only a decrease in the expression of the medium spiny neuron marker DARPP-32 (Jacobsen et al. 2010). The differences between full-length htt and N-terminal htt transgenic pigs provide further evidence for the toxicity of N-terminal mutant htt. Thus, as with HD mouse models, the expression of N-terminal mutant htt can cause robust neurological phenotypes and pathological changes in large animals. These studies also suggest that protein context and the length of htt fragments may determine the nature of the neuropathology. For example, exon1 (1–67 amino acids) mutant htt in monkey brains causes axonal degeneration, whereas N-terminal 208 amino acids of mutant htt in pig brains can mediate apoptosis; however, in transgenic pigs (Uchida et al. 2001; Baxa et al. 2013) expressing a larger mutant htt fragment and in transgenic HD sheep

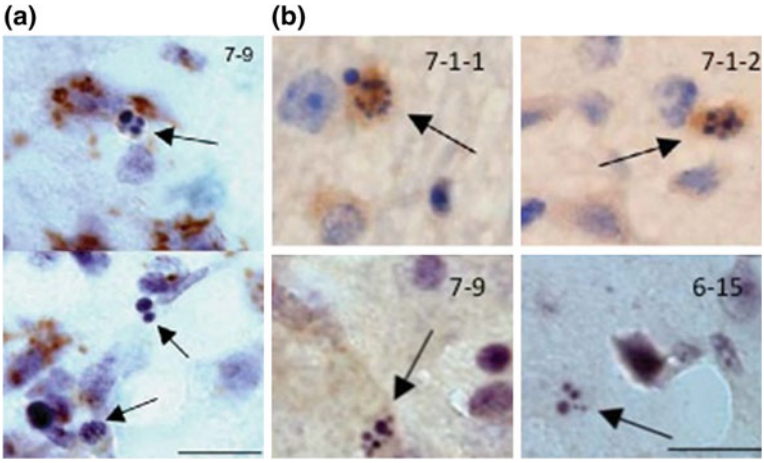

Fig. 2 Apoptotic cells in the brains of transgenic HD pigs. (**a**) Anti-polyQ (1C2) immunocytochemistry revealed the presence of mutant htt in the neurons of the brain striatal (*upper*) and cortical (*lower*) sections of HD transgenic pig (7-9). (**b**) Htt (EM48) immunocytochemistry also revealed apoptotic neurons (*arrows*) in transgenic HD pigs (7-1-1, 7-1-2, 7-9, and 6-15). Scale bars: 10 μm

(Jacobsen et al. 2010) expressing full-length (3,144 amino acids) mutant htt, there was no apoptosis, early animal death, or neurological phenotype reported. It is possible that neurodegeneration in large animals only occurs when sufficient degraded N-terminal fragments have accumulated in old animals. Thus, expressing N-terminal mutant htt fragments can facilitate disease progression, resulting in the early postnatal death of transgenic HD pigs and monkeys.

5 Insights from HD Models

It is clear that species differences play a critical role in the neurological phenotype differences in small and large animal models. There are considerable differences in development, life span, physiology, genetics, and anatomy between small and large animals (Table 1). An interesting issue is what the mechanisms behind these differences are. Certainly, there are a number of possible explanations. The short life span of mice is often believed to be responsible for the failure of HD mouse models to develop overt neurodegeneration. It is also possible that the misfolded form of N-terminal mutant htt is more toxic to the neuronal cells of pigs and monkeys than to rodent neurons. Considering that gestation in monkeys and pigs is much longer than in mice, this longer time period may allow overexpression of the toxic form of mutant proteins, such as N-terminal mutant htt, to cause more severe neurotoxicity in the pig and monkey brains. Also, because the brain circuitry in pigs and monkeys is more complex than in mice, this complexity may render

Table 1 Major differences in some species

Species	Sex maturity	Generation (day)	Life span (year)	Body weight (kg)
Human	15–18 years	266	75	50
Monkey (Rhesus)	3–5 years	165	25	6
Pig	9–11 months	114	7	80
Mouse	7 weeks	19–21	2	0.03

neurons in large animals more vulnerable to misfolded mutant htt. Finally, the cellular ability to cope with misfolded proteins during development and adulthood may be different between species. The rapid maturation of rodent neurons during early brain development may reduce their sensitivity to misfolded proteins, which can also explain why mouse models can survive to adulthood even when they express the same mutant htt N-terminal fragment.

Considering that HD and other neurodegenerative disease are age-dependent disorders, it is important to understand the differences in the aging process among different animal species when these animals are used to model neurodegenerative diseases. The life spans of these species differ drastically, indicating that aging processes in different species are not identical. Also, the early development of these mammalian species requires significantly different periods of time. For example, the gestation period for mice is 21 days, whereas pigs and monkeys require 4–5 months to reach full-term development. In addition to these significant differences, the anatomy, physiology, function, and circuitry of pig and monkey brains are much more complex than mouse brains (Table 1). These differences clearly indicate that monkeys and pigs are much closer to humans than mice and also explain why larger animal models would be better to mimic the pathological features seen in human patients. Indeed, a genetic pig model of cystic fibrosis replicates abnormalities seen in cystic fibrosis patients that do not occur in mouse models (Rogers et al. 2008). Of the large animals, monkeys are the best to model neurological diseases of humans, especially for cognitive behavioral analysis. Pigs, on the other hand, have a long life span (12–15 years), are easily bred, and reach puberty at 5–6 months, so they also offer advantages for biomedical research over other large animals, such as primates, for ethical and economic reasons (Lind et al. 2007).

6 Future Studies with Large Animal Models

Nevertheless, generation of genetic models using large animals is much more challenging than establishing genetic mouse models. So far, there are no ES cells from pigs, monkeys, or other large animals that can be used for generating gene-targeted animals. Induced pluripotent stem (iPS) cells are similar to embryonic cells and can be potentially used for altering endogenous genes and gene targeting in various species of animals. However, use of iPS cells for gene targeting is still

under development. Most work done on transgenic monkeys involved the use of lentiviral vector infection of fertilized oocytes and embryo transplantation (Yang et al. 2008; Sasaki et al. 2009; Niu et al. 2010), which requires a considerable number of donor and surrogate monkeys. Successful generation of transgenic pigs can also be achieved via nuclear transfer, a cloning strategy that has a low rate (<1–2 %) for transferred pig embryos to develop to term (Lai and Prather 2003). In addition, the costs in maintaining and breeding large animals as well as the ethical concerns and strict regulation of the use of large animals also make it difficult to use them for biomedical research.

Recent advances in gene targeting have opened new avenues for gene targeting in large animals. The new technology, called TALEN and cas9, enable gene targeting via transcription activator-like effector nucleases (TALENs) and the Cas9 endonuclease from the type II bacterial CRISPR/Cas system, respectively. Gene targeting via TALENs or Cas9 can occur in embryos without involving ES cells (Shen et al. 2013; Wang et al. 2013). The new technology involves the use of DNA binding peptides that can bind specific target DNA sequences to allow cleavage DNAs by nuclease, thus creating the loss of function of a specific gene. This approach can be applied to one-cell fertilized embryos to cause null mutations of specific genes in the body. It has been reported that replacement of an endogenous gene via Cas9-mediated DNA cleavage can also be achieved (Wang et al. 2013).

Although the rodent models of neurodegenerative diseases will remain as a major modeling system for investigating a variety of diseases because of the difficulty and expense of generating and characterizing large animals, large animal models will make a more rigorous system for validating the relevance of critical findings from small animal models. The large animal models will help us to address the following important issues. It would be interesting to know how mutant htt possesses neuronal toxicity in adult transgenic animals. Such studies would require new transgenic animal models that can survive to adulthood, which can be accomplished by using different transgenic vectors that express mutant htt at a lower level or in an inducible manner. To verify that N-terminal mutant htt, rather than its overexpression, is indeed toxic, it would also be important to use a knock-in approach to express N-terminal mutant htt at the endogenous level. Transgenic models using higher mammalian species or large animals to model important neurodegenerative diseases would give us deeper insight into the pathogenesis of neurodegenerative diseases. In addition, given the frequent failures when it comes to clinical trials of drugs that have been found to work in small animal models, transgenic large animals could yield a more reliable system for verifying therapeutic efficacy before moving to clinical trials.

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References

Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J, Miyanohara A, Nejime T, Klima J, Macakova M, Marsala S, Weiss A, Kubickova S, Musilova P, Vrtel R, Sontag EM, Thompson LM, Schier J, Hansikova H, Howland DS, Cattaneo E, DiFiglia M, Marsala M, Motlik J (2013) A transgenic minipig model of huntington's disease. J Huntington Dis 2:47–68

Bradford J, Shin JY, Roberts M, Wang CE, Li XJ, Li S (2009) Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. Proc Natl Acad Sci U S A 106:22480–22485

Chan AW, Chong KY, Martinovich C, Simerly C, Schatten G (2001) Transgenic monkeys produced by retroviral gene transfer into mature oocytes. Science 291:309–312

Cheng PH, Li CL, Chang YF, Tsai SJ, Lai YY, Chan AW, Chen CM, and Yang SH (2013). miR-196a Ameliorates phenotypes of Huntington disease in cell, transgenic mouse, and induced pluripotent stem cell models. Am J Hum Genet. In press.

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548

Dawson TM, Ko HS, Dawson VL (2010) Genetic animal models of Parkinson's disease. Neuron 66:646–661

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993

Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE et al (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195

Gusella JF, MacDonald ME, Ambrose CM, Duyao MP (1993) Molecular genetics of Huntington's disease. Arch Neurol 50:1157–1163

Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci 19:2522–2534

Harjes P, Wanker EE (2003) The hunt for huntingtin function: interaction partners tell many different stories. Trends Biochem Sci 28:425–433

Jacobsen JC, Bawden CS, Rudiger SR, McLaughlan CJ, Reid SJ, Waldvogel HJ, MacDonald ME, Gusella JF, Walker SK, Kelly JM et al (2010) An ovine transgenic Huntington's disease model. Hum Mol Genet 19:1873–1882

LaFerla, FM, and Green KN (2012) Animal models of Alzheimer disease. Cold Spring Harb Perspect Med 2

Lai L, Prather RS (2003) Production of cloned pigs by using somatic cells as donors. Cloning Stem Cells 5:233–241

Lee Y, Dawson VL, and Dawson TM (2012) Animal models of Parkinson's disease: vertebrate genetics. Cold Spring Harb Perspect Med 2

Li SH, Li XJ (1998) Aggregation of N-terminal huntingtin is dependent on the length of its glutamine repeats. Hum Mol Genet 7:777–782

Li SH, Li XJ (2004) Huntingtin-protein interactions and the pathogenesis of Huntington's disease. Trends Genet 20:146–154

Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10:137–144

Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK (2007) The use of pigs in neuroscience: modeling brain disorders. Neurosci Biobehav Rev 31:728–751

Matsuyama N, Hadano S, Onoe K, Osuga H, Showguchi-Miyata J, Gondo Y, Ikeda JE (2000) Identification and characterization of the miniature pig Huntington's disease gene homolog: evidence for conservation and polymorphism in the CAG triplet repeat. Genomics 69:72–85

Mattson MP (2000) Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 1:120–129

Menalled LB, Sison JD, Wu Y, Olivieri M, Li XJ, Li H, Zeitlin S, Chesselet MF (2002) Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. J Neurosci 22:8266–8276

Niu Y, Yu Y, Bernat A, Yang S, He X, Guo X, Chen D, Chen Y, Ji S, Si W et al (2010) Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector. Proc Natl Acad Sci U S A 107:17663–17667

Orr HT, Zoghbi HY (2007) Trinucleotide repeat disorders. Ann Rev Neurosci 30:575–621

Perutz MF, Johnson T, Suzuki M, Finch JT (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. Proc Natl Acad Sci U S A 91:5355–5358

Qin ZH, Gu ZL (2004) Huntingtin processing in pathogenesis of Huntington disease. Acta Pharmacol Sin 25:1243–1249

Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, Rogan MP, Pezzulo AA, Karp PH, Itani OA et al (2008) Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science 321:1837–1841

Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M, Tomioka I, Sotomaru Y, Hirakawa R, Eto T et al (2009) Generation of transgenic non-human primates with germline transmission. Nature 459:523–527

Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA et al (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet 8:397–407

Shen B, Zhang J, Wu H, Wang J, Ma K, Li Z, Zhang X, Zhang P, Huang X (2013) Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell Res 23:720–723

Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ et al (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567

Uchida M, Shimatsu Y, Onoe K, Matsuyama N, Niki R, Ikeda JE, Imai H (2001) Production of transgenic miniature pigs by pronuclear microinjection. Transgenic Res 10:577–582

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384

Wang CE, Tydlacka S, Orr AL, Yang SH, Graham RK, Hayden MR, Li S, Chan AW, Li XJ (2008) Accumulation of N-terminal mutant huntingtin in mouse and monkey models implicated as a pathogenic mechanism in Huntington's disease. Hum Mol Genet 17:2738–2751

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153:910–918

Wheeler VC, White JK, Gutekunst CA, Vrbanac V, Weaver M, Li XJ, Li SH, Yi H, Vonsattel JP, Gusella JF et al (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. Hum Mol Genet 9:503–513

Woodman B, Butler R, Landles C, Lupton MK, Tse J, Hockly E, Moffitt H, Sathasivam K, Bates GP (2007) The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain Res Bull 72:83–97

Xu QQ, Huang SS, Song M, Wang CE, Yan S, Liu XD, Gaetig MA, Yu SP, Li H, Li S, Li X-J (2013) Synaptic mutant Huntingtin inhibits synapsin-1 phosphorylation and causes neurological symptoms. J Cell Biol. In press

Yang D, Wang CE, Zhao B, Li W, Ouyang Z, Liu Z, Yang H, Fan P, O'Neill A, Gu W et al (2010) Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. Hum Mol Genet 19:3983–3994

Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, Snyder B, Larkin K, Liu J, Orkin J et al (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453:921–924

Yuan J, Yankner BA (2000) Apoptosis in the nervous system. Nature 407:802–809

Zhou H, Cao F, Wang Z, Yu ZX, Nguyen HP, Evans J, Li SH, Li XJ (2003) Huntingtin forms toxic NH2-terminal fragment complexes that are promoted by the age-dependent decrease in proteasome activity. J Cell Biol 163:109–118

Therapeutic Strategies for Huntington's Disease

Ladislav Mrzljak and Ignacio Munoz-Sanjuan

Abstract Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disease, caused by expansion of the CAG repeat in the huntingtin (*HTT*) gene and characterized pathologically by the loss of pyramidal neurons in several cortical areas, of striatal medium spiny neurons, and of hypothalamic neurons. Clinically, a distinguishing feature of the disease is uncontrolled involuntary movements (chorea, dyskensias) accompanied by progressive cognitive, motor, and psychiatric impairment. This review focuses on the current state of therapeutic development for the treatment of HD, including the preclinical and clinical development of small molecules and molecular therapies.

Keywords Huntington's disease · Huntingtin · CAG repeats · Kynurenine · PDE · HDACs · ASO · siRNA · Zinc-finger repressors · Dopamine · Glutamate · GABA · Adenosine

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L. Mrzljak · I. Munoz-Sanjuan (✉)
CHDI Management/CHDI Foundation Inc., Los Angeles, CA, USA
e-mail: ignacio.munoz@chdifoundation.org

L. Mrzljak
CHDI Management/CHDI Foundation Inc., Princeton, NJ, USA

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1 Introduction to Huntington's Disease

Huntington's disease (HD) is a devastating, autosomal dominant, neurodegenerative disorder, which is caused by a CAG triplet repeat expansion in exon 1 of the huntingtin (*HTT*) gene, encoding an abnormally expanded polyglutamine (polyQ) tract (The Huntington's Disease Collaborative Research Group 1993; Zuccato et al. 2010). HD is the most prevalent of all triplet repeat diseases. The CAG-repeat length (>35 CAGs) is inversely correlated with age at diagnosis of clinical symptoms (typically motor symptoms), accounting for 60–70 % of this variation (Langbehn et al. 2010). Although the disease can manifest at any age, clinically relevant symptoms begin in mid-life (35–50 years) and progression is slow, taking ~20 years to death. In juvenile cases of HD (<20 years of age, with CAG repeats typically >60) the disease is accelerated and symptoms include more severe bradykinesia, rigidity, seizures, and severe dementia with little to no chorea (Vonsattel and DiFiglia 1998).

Clinically, adult HD is characterized by motor, cognitive, and psychiatric disturbances. These include impairments in executive function, planning, and working memory, impulsivity, loss of attention, motivation and self-care, and deficits in movement control (chorea, dyskinesias). In later stages, the patients develop rigidity, bradykinesia, and dementia (Biglan et al. 2009; Duff et al. 2007, 2010; Sanberg et al. 1981). There is also loss of body weight and muscle bulk even when patients are on a high-calorie intake (Fischbeck 2001). By the time a patient is clinically diagnosed with onset of disease there is an estimated 60–80 % loss of the striatum (Margolis and Ross 2001). The medium spiny neurons of the dorsal striatum appear to be the most vulnerable, even though HTT is ubiquitously expressed. Difficulty in swallowing leading to choking, pneumonia, and complications with lack of mobility generally result in death.

HD prevalence varies around the world with most countries having 3–10 cases per 100,000 people. Studies in Japan and Africa have shown lower prevalence rates; among the lowest are found in black South Africans with 0.01 in 100,000 (Folstein et al. 1987; Hayden et al. 1980; Wright et al. 1981). However, given an aging population and the inverse correlation between age at motor onset and length of the CAG, the current prevalence is expected to be considerably higher than

previously estimated (Hayden et al. 1980). In addition, in some large Latin American clusters (Colombia, Brazil, Peru, and Venezuela), the prevalence of the disease can be a staggering 10 % of the population (Wexler et al. 2004).

The discovery of the disease-causing mutation in *HTT* and the development of genetically engineered rodent models has enabled investigation of the potential pathogenic mechanisms in HD, through pharmacological/molecular intervention as well as genetic manipulation (Hult et al. 2010). Most of these transgenic animal models have large (>70) CAG repeats, similar to the repeat lengths common in juvenile HD; adult-onset HD is typically due to CAG repeat lengths in the low-to-mid 40s (Figiel et al. 2012; Switonski et al. 2012). Mouse models with a more physiological adult-onset CAG-repeat length develop few or no symptoms (aggregation of HTT, behavioral alterations), although more sensitive approaches to evaluating dysfunction need to be explored in aged animals. One of the greatest challenges in neuroscientific drug discovery, including HD, is the uncertainty of the predictive value of rodent models to clinical pathophysiology, and we must recognize their limitations and understand which pathways or mechanisms correlate to the human pathophysiology. As discussed previously, this would require a much more extensive effort to dissect pathogenic mechanisms during longitudinal studies in HD subjects, and to compare juvenile HD (caused by large expansions in HTT) and adult-onset HD (Munoz-Sanjuan and Bates 2011).

The mechanisms associated with HD pathogenesis share similar biology with other neurodegenerative diseases, and in general we lack a deep understanding of the specificity of the molecular networks triggered by continuous expression of mutant HTT. We have only recently begun to dissect molecular alterations in the various cells types specifically affected in HD, through genetic, molecular, and optogenetic techniques. Mechanisms implicated in HD include those relevant to DNA repair, transcriptional and translational modulation of expanded trinucleotide repeats (including somatic expansion), mitochondria and energy homeostasis, vesicular trafficking dynamics, oligomerization of mHTT (chaperone biology), autophagy, and synaptic signaling (Cattaneo et al. 2005; Hult et al. 2010; Munoz-Sanjuan and Bates 2011). Although these mechanisms are of significant interest, most studies have not provided sufficient molecular understanding of the dysfunction to enable a drug discovery program. Existing therapeutic programs target either HTT itself or its aggregation, or mechanisms thought to play a major role in progression, and which are amenable for traditional drug development: drugs aimed at restoring the circuitry changes observed in HD, and thought to underlie its symptomatology, or drugs aimed at restoring energetic deficits, autophagy induction in brain cells, or deficits in BDNF/TrkB signaling. Ongoing clinical trials are given in Table 1. Preclinical-stage therapeutic approaches are described in more depth in Table 2.

Table 1 Ongoing randomized clinical trials in HD (2013)

Clinical trial name or sponsor	Intervention	Size	Starting date	Current status
CREST-E	Creatine	650	2008	Recruiting
2CARE MGH	Coenzyme Q10	608	2008	Completed
MIG-HD	Fetal transplantation	60	2008	Completed
TREND-HD Amarin Neuroscience Ltd.	Ethyl-EPA (Miraxion™)	300	2005	Completed
NEUROHD	Tetrabenazine olanzapine tiapride	180	2008	Completed
Teva pharmaceutical industries	ACR16	437	2008	Completed
Heinrich-Heine University (Germany)	DBS of the globus pallidus	6	2009	Completed
Raptor	Cysteamine	98	2010	Completed
Charite University (Germany)	Buproprion	90	2010	Recruiting
REACH2	PBT2	100	2011	Completed
University of British Columbia	Memantine	25	2011	Recruiting
Charite University (Germany) ETON study	Epigallocatechin gallate	54	2011	Recruiting
GW Pharmaceuticals Ltd.	Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD)	25	2011	Completed
Auspex	SD-809 ER	100	2013	Recruiting
Pfizer	PF-02545920	65	2013	Recruiting

Completed studies included have not reported findings. Trials are shown in order of starting date

2 Modulation of Huntingtin Expression: Molecular Therapeutics

HTT is a soluble protein that is ubiquitously expressed with a biological function that is not well understood in the adult. HTT loss of function is lethal during early mouse development, so it is likely that HTT functions can be elucidated using developmental systems (Cattaneo et al. 2005; Munoz-Sanjuan and Bates 2011; Reiner et al. 2003; Woda et al. 2005). HTT is a 3,144 amino acid protein with a polyglutamine stretch and a polyproline domain located in the N-terminus, nuclear import and export signals, numerous regions predicted to form highly structured HEAT repeats, and novel recently identified aggregation motifs outside the polyglutamine tract itself (Andrade and Bork 1995). HTT can be found in the cytosol, in cell membranes (with a strong association with the endoplasmic reticulum and Golgi apparatus), and in the nucleus. A pathological hallmark of HD is the presence of intranuclear and neuropil inclusions composed of aggregated N-terminal HTT fragments and a variety of ubiquitinated proteins (Becher et al. 1998; Martin-Aparicio et al. 2001; Yamamoto et al. 2000).

Table 2 Preclinical therapeutic programs at CHDI, past and present

Target/agent	Status	Partners/comments
KMO inhibitor	Active	Evotec
HDAC Class II inhibitor	Active	BioFocus
HTT Antisense Oligonucleotides	Active	ISIS/Roche
HTT siRNAs	Active	Alnylam/Medtronic
HTT Zinc-finger repressor (ZFPs)	Active	Sangamo/Shire
HTT miRNA	Active	Genzyme/Sanofi
HTT small-molecule modulator	Active	BioFocus
TrkB agonist monoclonal antibody	Active	Pfizer
BDNF protein (AAV)	Active	
JNK3 inhibitor	Active	AMRI
PDE2 inhibitor	Active	Pfizer
PDE9 inhibitor	Active	Pfizer
PDE10 inhibitor	Active	Pfizer
PDE4D negative allosteric modulator	Active	deCode genetics
p38 inhibitor	Active	POC molecule testing
Flupirtine	Active	POC molecule testing
GlyT1 inhibitor	Active	POC molecule testing
M4 potentiator	Active	POC molecule testing
P2X7 inhibitor	Active	Lundbeck POC molecule testing
Myostatin neutralizing monoclonal antibody	Active	Pfizer POC molecule testing
5HT6 antagonist	Active	POC molecule testing
H3 antagonist	Active	POC molecule testing
mGluR2/3 potentiator	Active	POC molecule testing
Hsp90 inhibitor	Discontinued	Kings College London
A2a inhibitor	Discontinued	POC molecule testing
TG2 inhibitor	Discontinued	Evotec
mGluR5 antagonist	Discontinued	Roche POC molecule testing
Caspase-6 inhibitor	Discontinued	Evotec
Caspase-2 inhibitor	Discontinued	Evotec
PDHK inhibitor	Discontinued	AMRI
GLT-1/EAAT2 activator	Discontinued	Evotec
Sirtuin-1 activator	Discontinued	BioFocus

POC molecule testing: proof-of-concept tool molecule used to query target in R6.2 or Q175 mice

Given the monogenic nature of HD, lowering levels of mutant HTT is predicted to alleviate disease burden, and this appears substantiated by data in rodent models of HD (Kordasiewicz et al. 2012; Martin-Aparicio et al. 2001; Yamamoto et al. 2000). HD patients with homozygous expansions do not seem to manifest increased severity or accelerated progression of the disease (Wexler et al. 2004), although in rodents there is a clear HTT dose-dependent alteration in symptom onset and progression (Crook and Housman 2011; Figiel et al. 2012; Heikkinen et al. 2012; Switonski et al. 2012). In hypomorphic mice, transgenic mice with

inducible expression of mutant *HTT*, or mice expressing inducible shRNAs targeting *HTT* expression, greater than 70 % knockdown of both alleles is toxic in embryonic and adult contexts. However, additional studies in adult mice and in nonhuman primates (NHPs) locally administered with HTT-lowering therapeutics have demonstrated that the reduction of both alleles to 50 % levels for an extended period of time appears to be well tolerated (Grondin et al. 2012; Hilditch-Maguire et al. 2000; Kordasiewicz et al. 2012; Woda et al. 2005).

Given that the function of HTT is unclear and that it lacks domains amenable to modulation by traditional small-molecule therapeutics, the main approach to slow disease progression has centered on decreasing HTT expression, and four distinct clinical approaches are being pursued. Lowering of HTT mRNA, mostly in a nonallele selective manner, is being pursued via a variety of strategies, including antisense oligonucleotides (ASOs; ISIS Pharmaceuticals; and Roche), small interfering RNAs (siRNAs; Alnylam; and Medtronic), or micro RNAs (miRNAs; Genzyme; and Sanofi-Aventis) (Grondin et al. 2012; Sah and Aronin 2011; Stiles et al. 2012). In addition to these RNA-targeting modalities, a DNA-targeting strategy is being developed by Sangamo and Shire, via the development of CAG-directed zinc-finger repressor (ZFP) proteins. All these programs are in late preclinical development stages. We are working with these partners to enable clinical development for all these programs, although our current efforts at the moment are directed toward the development and validation of HTT-dependent biological readouts of the effectiveness of the therapeutics by focusing on detection methods for HTT levels, or early alterations after HTT suppression which can be applied in a clinical setting as early measures of drug-induced effects.

The RNA-directed therapeutics target regions of the mRNA that are downstream of the pathogenic exon1 sequences. A recent study shows that a mis-spliced form of HTT message is transcribed in animals and in human tissues, and is predicted to generate a truncated amino-terminal fragment of HTT, with presumed enhanced toxicity (Sathasivam et al. 2013). This alternatively spliced HTT mRNA would not be modulated by existing RNA-directed therapeutics, which could therefore limit their effectiveness if indeed this mRNA is important in disease pathogenesis. In contrast to the RNA-directed therapies, using DNA-directed ZFPs would not be affected by this finding. Similarly, second generation RNA therapies targeting the expanded CAG tract would also target this readthrough spliced isoform of HTT.

The various modalities in clinical development, expected to reach patients in the next 24 months, also differ in their delivery to areas thought to be important for disease progression: ISIS and Roche will deliver the ASO therapeutic via an intrathecal route, aiming to lower HTT expression in cortical areas, which has been shown to be important for symptom modification in transgenic animals (Gu et al. 2005); the Medtronic siRNA will be delivered to the putamen via a surgically implanted pump and catheter; and the ZFPs and miRNAs will be delivered via viral vectors, most likely adeno-associated virus (AAV). A critical difference between the delivery approaches is that, since AAVs integrate into the host genome, transduction needs to take place only once and a lifetime expression of the

HTT-suppressing agent is envisioned. As far as we are aware, these therapeutic agents will be delivered to the caudate-putamen region. In Parkinson's disease trials with GDNF and neurturin, AAV delivery has been well tolerated and transgene expression appears stable several years after initial transduction (Bartus et al. 2013).

2.1 Biomarker Strategies to Enable Clinical Trials for Molecular Therapeutics

A major outstanding question for the clinical development of the HTT-directed molecular therapeutics is the assessment of proximal alterations to disease progression that reflect a change in mutant (or total) HTT protein levels in a region-specific manner. Ideally, in Phase II trials, evidence of proof of mechanism needs to be determined. Traditionally, pharmacodynamic endpoints are incorporated in Phase II to validate that the administered drug is able to modulate the target or biological mechanism of interest. This is challenging in the context of a protein (HTT) whose expression is the desired endpoint.

To address these challenges we are exploring endpoints that will assess lowered HTT levels, or an evaluation of biological endpoints proximal to HTT itself. CHDI, in collaboration with BioFocus, has developed a panel of ELISA-based Meso Scale Discovery (MSD) electro-chemiluminescence assays to enable the detection of nonexpanded and polyQ-expanded primate and rodent HTT in a variety of cells and tissue samples. These assays are highly sensitive (detecting fmols of protein) and robust, and can detect HTT proteins in complex tissue samples. These set of assays (manuscript in review) add to the existing TR-FRET-based assays developed by Novartis (Baldo et al. 2012; Weiss et al. 2012). Recently, Novartis in collaboration with CHDI and UCL have applied the TR-FRET assay to detect mHTT in peripheral human monocytes, enabling HTT measurements for the development of therapeutics with a peripheral effect on HTT levels (Baldo et al. 2012; Weiss et al. 2012).

As in the Alzheimer's disease field, we also initiated a program to develop imaging agents that can bind selectively to HTT to visualize, noninvasively, HTT levels in the brain. Some of these small molecules display nanomolar affinities for mutant HTT and are being developed to enable a Phase II study in HD. Other imaging modalities are being explored to understand whether changes in biological endpoints subject to modulation via mutant HTT levels can be incorporated into clinical trials. For instance, one of the most conserved alterations in HD patients and all HD animal models is the downregulation of several neurotransmitter receptors and enzymes critical for basal ganglia function, such as the dopamine signaling components (D1, D2 receptors, and the DAT transporter) (Andre et al. 2010; Brandt et al. 1990), the cannabinoid receptor CB1 (Van Laere et al. 2010), the enzyme PDE10 (Giampa et al. 2010; Sadri-Vakili et al. 2007; Sugars 2003),

and the metabotropic glutamate receptor (mGluR5) (Ribeiro et al. 2010), all of which can be visualized with existing, clinically used, PET or SPECT tracers.

Recent evidence suggests that lowering mutant HTT, even late in disease progression, leads to an increase in the expression of some of these genes, which can presumably be clinically monitored by imaging agents. The existing imaging tracers can therefore be used as indirect markers of mHTT lowering. Additional technologies under consideration to monitor early signs of disease modification will likely include noninvasive assessment of metabolic measurements, such as FDG-PET, or of sensitive measures which can monitor changes in the underlying, altered, basal ganglia circuitry, such as qEEG or fMRI measurements (Eidelberg and Surmeier 2011; Gray et al. 2013; Hunter et al. 2010; Wolf et al. 2013).

Due to the challenges of central delivery, distribution, and safety of the molecular therapies, CHDI has recently embarked on a small-molecule HTT expression modulation program. The recent development of quantitative assays to monitor wildtype and mutant HTT expression levels has enabled the use of these assays in high throughput screens to identify small molecules or genes which can modulate HTT expression. Small molecules targeting novel mechanisms such as RNA-stability or translational efficiency of the expanded HTT mRNA (perhaps distinguishing CAG tract length) might be identified in such a screen. The hits from these screens will need to be deconvoluted to understand the mechanism of action, selectivity over other proteins, and their putative molecular targets.

3 Small-Molecule Therapeutics

A key challenge in translational research is prioritizing—with scant data from human studies—mechanisms relevant to human disease pathogenesis. To identify the mechanisms that can significantly slow disease progression, CHDI has formed five biological mechanism teams to design, oversee, and implement key preclinical and clinical experiments to investigate mechanistic hypotheses in HD. These biological mechanisms include autophagy/clearance of HTT and energetic and synaptic mechanisms, including the modulation of TrkB signaling. In this review, we only cover strategies in advanced stages of preclinical development or in the clinic, so the approaches targeting autophagy in the brain are not described.

Alterations in HD synaptic function may be responsible for many of the early symptoms of HD. Normal HTT interacts with various cytoskeletal and synaptic vesicle proteins essential for exocytosis and endocytosis (Caviston and Holzbaur 2009; Qin et al. 2004), and altered interactions of mHTT likely contributes to abnormal synaptic transmission in HD. Synaptic alterations in corticostriatal transmission have been well documented and are one of the earliest changes detected in rodent HD models (Figiel et al. 2012; Heikkinen et al. 2012; Switonski et al. 2012). In addition, both in rodent models and in numerous clinical studies, neurotransmitter receptors critical to the function of the affected circuitry display altered levels, such as dopamine and cannabinoid receptors (Cattaneo et al. 2005;

Munoz-Sanjuan and Bates 2011). Although initial reports focused on the disturbances and cell death of striatal medium spiny neurons (MSNs) it is now apparent that significant alterations to striatal interneurons exist that are likely to play a very significant role in synaptic pathogenesis (Cepeda et al. 2013; Eidelberg and Surmeier 2011; Horne et al. 2013; Pisani et al. 2007; Vlamings et al. 2012). In addition, ongoing efforts to refine our understanding of the molecular and neurochemical alterations in cortico- or thalamo-striatal connectivity, as well as the physiological alterations in the output nuclei of the basal ganglia, will lead to the identification of novel targets for intervention, with the aim of understanding (and restoring) time-dependent alterations in this circuitry.

We and others have evaluated the neurochemical and physiological alterations that develop over time in HD rodent models by analyzing neurotransmitter alterations and changes in neuronal firing in the affected circuitry in vivo as the disease develops, and in slice preparations derived from the affected circuitry during disease progression (Cepeda et al. 2013; Heikkinen et al. 2012; Andre et al. 2010). As part of this broad effort to 'map' the alterations in synaptic physiology in an HD context and the system's responsiveness to pharmacological or electrical manipulations, we are testing a large set of clinical-stage molecules known to modulate the underlying circuitry, such as agents able to query pathways modulated by glutamate, GABA, histamine, monoamines, dopamine, and cannabinoids in the basal ganglia. Some of these molecules have been developed for the treatment of HD in collaboration with pharmaceutical companies (such as selective inhibitors of phosphodiesterases (PDEs) 2, 9, and 10 in collaboration with Pfizer; see below) or exclusively as CHDI-driven projects (such as inhibition of kynurenine monooxygenase). Much of the ongoing preclinical research at our collaborating laboratories testing various clinical candidates are part of an ongoing scientific and clinical collaboration between CHDI and industry, reflecting a change in the willingness of pharmaceutical companies to actively develop therapeutics for HD.

4 Synaptic Mechanisms

4.1 Existing and Experimental Therapeutics Targeting the Dopaminergic System

Antidopaminergic drugs such as tetrabenazine and antipsychotics are still the most frequent therapy in the management of hyperkinetic (chorea) or psychiatric symptoms in HD (Chen et al. 2012; Frank 2010; Ross and Tabrizi 2011).

4.1.1 Tetrabenazine

First introduced in the 1970s to treat hyperkinetic movement disorders, tetrabenazine became the first FDA-approved drug to treat HD more than 30 years later (Frank 2010). Tetrabenazine inhibits the vesicular monoamine transporter 2 (VMAT2) that is predominantly localized in the brain (Paleacu 2007; Pettibone et al. 1984a, b), preventing monoamine transport from the cytoplasmic pool into the synaptic vesicles and enhancing their catabolism, with more selectivity to dopamine than norepinephrine. (Pettibone et al. 1984a, b) Two features distinguish tetrabenazine from the known VMAT inhibitor reserpine; tetrabenazine is a reversible VMAT inhibitor in contrast to the irreversible inhibition of reserpine, and tetrabenazine is highly selective for centrally localized VMAT2 while reserpine targets both peripherally localized VMAT1 as well as VMAT2 (Paleacu 2007). These tetrabenazine features result in a much shorter action than reserpine (hours vs. days) and avoid reserpine's major peripheral side effects, such as orthostatic hypotension and diarrhea (Frank 2010; Mehvar et al. 1987; Paleacu 2007; Roberts et al. 1986).

Two randomized, double-blind, placebo-controlled studies were crucial for the approval of tetrabenazine for HD in the US: the TETRA-HD study conducted by the Huntington Study Group (HSG) that evaluated treatment of chorea (Huntington Study Group 2006) and a study by Frank et al. (2008), that evaluated the reemergence of chorea after discontinuation of tetrabenazine dosing. The TETRA-HD study found a significant reduction of chorea score in the Unified Huntington's Disease Rating Scale (UHDRS) versus placebo, and Frank et al. found the worsening of chorea score upon tetrabenazine withdrawal (Frank 2010). However, while improving chorea, tetrabenazine leads to numerous side effects due to its mechanism of central inhibition of the monoaminergic system. The major side effects during the treatment-titration phase include insomnia, somnolence and depressed mood, sedation, anxiety, Parkinsonism, fatigue, akathisia, drowsiness, and potential cognitive impairment during the chronic treatment phase (Frank 2010). Therefore, there is still a need for small molecules and other approaches to manage hyperkinetic symptoms.

Currently, CHDI in collaboration with academic and pharmaceutical industry collaborators is exploring therapies focused on the modulation of the hyperexcitability of the direct D1 pathway in the basal ganglia with small molecules like M4 muscarinic receptor positive allosteric modulators and KCNQ2/3 channel openers (M-current modulation), as well as deep brain stimulation (DBS) approaches (see below and Table 2). Modulation of glutamatergic neurotransmission by mGluR5 and Group II metabotropic glutamate receptor (mGluR2/3) agonists, alone or in combination with neuroleptics, may be another alternative given the evidence in rodent HD models of a beneficial effect of metabotropic glutamate receptor signaling modulators (Doria et al. 2013; Reiner et al. 2012; Ribeiro et al. 2010; Schiefer et al. 2004).

4.1.2 ACR16 (Pridopidine)

ACR16 targets the dopamine D2 receptor and is currently in clinical development for treatment of HD (Lundin et al. 2010). In vitro the drug behaves as a low affinity, low efficacy partial agonist with fast dissociation properties (Dyhring et al. 2010; Kara et al. 2010). In vivo the drug is described as a "dopamine stabilizer" since in rodents it normalizes hyperdopaminergic states and reduces hyperlocomotion induced by amphetamine, but preserves spontaneous locomotor activity and has stimulatory action in the habituated states where the dopaminergic tone is considered to be low (Ponten et al. 2010; Rung et al. 2008). ACR16 also reverses the increased locomotor behavior induced by the NMDA antagonist MK-801 and elevates the levels of dopamine and norepinephrine in the cortex, striatum, and nucleus accumbens (Ponten et al. 2010). It is hypothesized that state-dependent stabilization of psychomotor activity by ACR16 is the result of dual mechanistic action; antagonism of dopamine D2 receptors and strengthening of cortical glutamate functions that can be important in restoring the cortical-subcortical functional neuronal circuitry impaired in HD (Ponten et al. 2010). ACR16 was not tested in HD animal models, however, its efficacy and safety was evaluated in a small Phase II (Lundin et al. 2010) and the larger Phase III randomized, double-blind, placebo-controlled MERMAiHD study (De Yebenes et al. 2011; Squitieri et al. 2013). In these studies ACR16 was well tolerated up to 90 mg/day with a safety profile similar to placebo and, based on the Phase II study indicating that ACR16 improved voluntary motor function, efficacy was evaluated in Phase III using the modified motor score as the primary endpoint. This endpoint was not met. Currently, ACR16 efficacy is being evaluated by Teva Pharmaceuticals in an open label study (OPEN-HART, ClinicalTrials.gov Identifier NCT01306929) where motor function is evaluated using the UHDRS Total Motor Score as the primary endpoint.

4.2 Modulation of Glutamate Signaling Pathways

The excitatory neurotransmitter glutamate plays a crucial role in the modulation of neuronal circuitry of the basal ganglia affected in HD. In particular, glutamate is the major neurotransmitter in parts of that circuitry such as the cortico-striatal-thalamo-cortical and subthalamic nucleus efferent systems (Andre et al. 2010). A current working hypothesis in HD is that increased levels of glutamatergic neurotransmission in the striatum are neurotoxic for MSN neurons. This theory is termed the excitotoxicity hypothesis of HD, and has been a major driver in the development of HD therapeutic strategies in the past (Andre et al. 2010; Munoz-Sanjuan and Bates 2011). Based on this theory, antiglutamatergic therapies may be beneficial in HD (Andre et al. 2010; Venuto et al. 2012). A major hurdle in developing glutamatergic-targeted HD therapeutics is a lack of mechanistic understanding of glutamatergic neurotransmission in the HD brain, including tonic

levels of glutamate, potential alterations in glutamate release mechanisms, level, localization and function of ionotropic and metabotropic glutamate receptors, and alterations in glial glutamate uptake. CHDI is committed to addressing these issues and is currently developing methods to measure extracellular levels of glutamate in the human brain based on metabotropic glutamate receptor occupancy using PET (Miyake et al. 2011).

The most reproducible finding of glutamatergic signaling impairment in both HD animal models and HD patient postmortem brains is a downregulation of glutamate uptake (Hassel et al. 2008; Miller et al. 2012) and glutamate transporter GLUT1 (EAAT2) expression localized to glial cells (Faideau et al. 2010; Miller et al. 2012). These findings suggest dysregulation of extracellular glutamate levels in HD, and point to GLUT1 as a potential therapeutic target. Indeed, in the R6/2 mouse model, chronic treatment with the antibiotic ceftriaxone upregulates GLUT1 expression and improves glutamate uptake (Miller et al. 2012; Sari et al. 2010). However, to date, there are no small molecules that increase GLUT1 function in HD and are suitable for drug development since the target of ceftriaxone is unknown. Similarly, whether the loss of GLUT1 expression is compensatory or causative is unknown; molecular or genetic approaches are likely required to determine whether an increase in activity or expression of GLUT1 is sufficient to ameliorate disease pathology in HD rodent models.

Antiglutamatergic therapies, such as the antagonism of NMDA receptors, are being explored clinically using amantadine, ramacemide, and memantine (Huntington Study Group 2001) (See ClinicalTrial.gov). The antiglutamate release agent riluzole failed to modulate HD symptoms in a clinical study (Landwehrmeyer et al. 2007). Other approaches might be explored therapeutically given initial evidence in rodent models that modulation of NR2B, mGluR2/3, and mGluR5 signaling appears to show beneficial effects in at least one rodent model of HD (Doria et al. 2013; Milnerwood et al. 2012; Reiner et al. 2012; Ribeiro et al. 2010; Schiefer et al. 2004). However, given that the models show a biphasic alteration in glutamate signaling in corticostriatal synapses, the timing of administration of these agents makes their development challenging. Similarly, the interplay between tonic glutamate and GABA signaling in the basal ganglia circuitry needs to be explored in more depth. Novartis sponsored a Phase II trial in HD with their mGluR5 antagonist AFQ056, but this study has been terminated (ClinicalTrials.gov identifier NCT01019473).

Future and ongoing experimental medicine studies by CHDI and academic/industrial collaborators to better understand the glutamatergic system in HD may facilitate development of glutamatergic therapies, such as modulation of glutamate release via GPCR receptors localized at glutamatergic synapses such as cannabinoid 1 (CB1), histamine H3, muscarinic M4, and serotonin 5HT6 receptors.

4.3 Cannabinoid and Adenosine Signaling

The importance of endocannabinoid signaling in the basal ganglia circuitry is well-known (Cachope 2012; Skaper and Di Marzo 2012). Imaging and histological studies have highlighted a decrease of CB1 expression in the lateral and medial striatum of HD patients (although not in the cortex), and in HD rodent models there is a loss of CB1 expression in MSNs and a subset of interneurons of the striatum, suggesting that altered endocannabinoid signaling might be relevant to the psychiatric, cognitive, and motor symptoms of HD (Allen et al. 2009; Bari et al. 2013; Chiodi et al. 2012; Denovan-Wright and Robertson 2000; Glass et al. 1993, 2000; Horne et al. 2013; Lastres-Becker et al. 2002; Richfield and Herkenham 1994). The CB1 receptor is one of the earliest downregulated transcripts in most HD models, similar to the alterations observed for D2, A2a, and PDE10, suggesting a primary role for these signaling pathways in the effects of mHTT expression (Blazquez et al. 2011; Chiodi et al. 2012; Dowie et al. 2009, 2010; Heikkinen et al. 2012; Horne et al. 2013; Van Laere et al. 2010).

A recent study explored the chronic administration of CB1-activating drugs, including URB597 (a fatty acid amide hydrolase (FAAH) inhibitor), delta-9-tetrahydrocannabinol (THC), or the CB1 agonist HU210 in R6.1 mice, an HTT-fragment model of HD (Dowie et al. 2010). These compounds were relatively well tolerated, with the exception of increased seizures. Treated animals showed few changes in disease phenotypes, including a lack of effect in the rotarod, Y-maze, or open-field tasks. Intriguingly, ubiquitin-quantification of HTT aggregates in these mice showed increased aggregation (or ubiquitination) after chronic dosing with URB597 (Huntington Study Group 2006). Unfortunately, in this study the authors did not explore the electrophysiological or neurochemical effects of their treatments in the HD mice, although they did show a partial restoration of CB1 binding and expression by URB597 (but not with direct CB1 agonists), suggesting that FAAH inhibition might partially restore some of the deficits routinely observed in HD rodent models. Another study, conducted in R6.2 mice, showed that THC administration before disease onset attenuated motor deficits, synaptic marker decrements, striatal volume loss, BDNF expression, and HTT aggregation in this model (Blazquez et al. 2011).

However, given the fact that endocannabinoid signaling affects motor output, and that the models are mostly hypokinetic (as opposed to the hyperkinetic presentation typical of adult-onset HD), the evaluation of this mechanism in HD rodent models with large CAG expansions might not reflect its therapeutic potential for HD. A detailed investigation of the role of cannabinoid signaling in electrophysiological or circuitry aspects of the disease, rather than behavior, might be more informative.

The role of CB1 in HD pathogenesis has also been explored genetically by crossing a CB1 knockout mouse to the R6.2 and N171-Q82 models of HD. Results of these studies show that the deletion of CB1 exacerbates disease progression, suggesting that HD progression might be a consequence of, or influenced by,

decreased CB1 signaling (Blazquez et al. 2011; Mievis et al. 2011). Importantly, in addition to the detrimental effects of CB1 loss in motor tasks in R6.2 mice, there was an increase in aggregate formation and an enhancement of striatal volume loss (Blazquez et al. 2011).

Another relevant aspect of cannabinoid signaling is the known intersection between CB1 signaling and BDNF expression and signaling. While CB1 activity can influence the expression levels of BDNF itself (Blazquez et al. 2011) and may contribute to the loss of BDNF expression observed in the HD brain, BDNF signaling can also affect release of both GABA and endocannabinoids in the brain (Goggi et al. 2002; Lemtiri-Chlieh and Levine 2010). Since a beneficial role of BDNF/TrkB signaling in HD has been well documented (see below), it is plausible that some of these effects are mediated by a restoration of CB1 signaling, and its effects in neurotransmission and information processing in the basal ganglia. The interplay between BDNF and CB1 is under active investigation at CHDI and several academic laboratories.

Although the effects of cannabinoid administration in HD patients have not been rigorously explored clinically, a recent double-blind randomized, cross-over study with 22 patients evaluated the effects of nabilone, a synthetic analog of THC, in HD symptoms. The study, not sufficiently powered to assess efficacy, demonstrated that nabilone has an acceptable safety profile and significantly improved psychiatric endpoints, as well as a marginal improvement in the chorea subscore of the UHDRS (Curtis et al. 2009). A second study using two natural cannabinoids is underway (Table 1), although the results from this study have not yet been reported. Given the alterations in this signaling mechanism, and its importance in basal ganglia function, the role of this neuromodulator in HD should be further investigated.

The adenosine receptor 2a (A2a) has also been shown to be downregulated prior to HD symptom onset. Despite the loss of expression of A2a, its signaling appears to be preserved in HD models (Chou et al. 2005; Dowie et al. 2009; Glass et al. 2000; Orru et al. 2011a; Popoli et al. 2008; Tarditi et al. 2006). A2a receptors are enriched in indirect-pathway striatopallidal MSNs, where they modulate dopamine D2 receptor signaling via the activation of cAMP. A2a signaling mechanisms are complex, tied to the pre versus postsynaptic localization of the receptor, where it forms heteromeric complexes with other receptors, such as D2 and mGluR5 in the postsynaptic site, or adenosine A1 receptors in the presynaptic compartment (Orru et al. 2011a, b; Popoli et al. 2008). Because of these complexes, the activity of A2a receptor modulation is difficult to dissect in terms of pre versus postsynaptic effects. For instance, presynaptic inhibition of A2a/A1 heteromers decreases presynaptic glutamate release, which presumably might be of benefit in HD if the excitotoxicity hypothesis is indeed correct.

Inhibition with the A2a antagonist KW-6002 (istradefylline) induces a hyperlocomotor phenotype in rodents when dosed acutely, although this effect is lost in a transgenic HD rat model at later stages, suggesting a loss of postsynaptic A2a-mediated signaling. In this same study, presynaptic signaling was preserved in the HD rat model, arguing for a selective alteration in postsynaptic MSN A2a

signaling (Orru et al. 2011a). In HD mouse models, several reports suggest that both the inhibition and activation of A2a receptors are beneficial (Chou et al. 2005; Domenici et al. 2007; Ferrante et al. 2010; Martire et al. 2010; Popoli et al. 2008). However, the acute locomotor effects of A2a antagonists in these (mostly hypokinetic) rodent models might give a false impression of a beneficial effect. We have shown that the positive motor findings of KW-6002 in R6.2 mice are driven by the acute effects of the drug, and that chronic dosing does not ameliorate disease phenotypes if the animals are tested when the drug is not on board (unpublished observations). In this regard, Domenici et al. found that a brief exposure to SCH58261, an A2a antagonist, early in the postnatal period has sustained effects several weeks after compound administration, so it is possible that the effects of A2a antagonism are significant in disease progression only during a certain window of striatal maturation (Domenici et al. 2007).

Several A2a antagonists have been developed for the treatment of Parkinson's disease. Clinical development of these molecules was stopped due to lack of efficacy or adverse side effects including dyskinesias, which is a significant concern for HD patients (Hauser et al. 2011; Mizuno et al. 2010). The development of this class of agents for the treatment of HD, therefore, necessitates a clearer understanding of the role of the multitude of A2a containing receptors and their influence, so that these potentially important side effects can be mitigated while retaining some of the potential beneficial effects of the postsynaptic receptor modulation.

4.4 Deep Brain Stimulation Strategies

Based on the success of the deep brain stimulation (DBS) in the treatment of movement disorders like Parkinson's disease (Deuschl et al. 2006), experimental DBS was applied to attenuate hyperkinetic motor symptoms in HD patients (Fawcett et al. 2005; Fasano et al. 2008; Hebb et al. 2006; Huys et al. 2013; Kang et al. 2011; Moro et al. 2004; Tang et al. 2005; Temel et al. 2006; Velez-Lago et al. 2013). In HD there is an apparent early loss of striatopallidal D2-positive MSNs in the striatum that leads to the attenuation of striatal inhibitory activity in the external segment of the globus pallidus (GPe), increased GPe firing and reduced activity in the subthalamic nucleus (STN) resulting in decreased firing of neurons in the internal segment of the globus pallidus (GPi). Consequently, decreased neuronal activity in the GPi results in increased thalamic and cortical activity and emergence of chorea (Albin et al. 1989). Therefore, the target of DBS therapy in HD has involved the bilateral stimulation of the GPi (Fasano et al. 2008; Fawcett et al. 2005; Hebb et al. 2006; Kang et al. 2011; Moro et al. 2004; Velez-Lago et al. 2013) although in one report the GPe was also targeted (Ligot et al. 2011). The frequencies used for brain stimulation were between 40 and 180 Hz, with the best results obtained with 130 Hz. These case studies reported improvement of chorea (Fasano et al. 2008; Fawcett et al. 2005; Hebb et al. 2006; Huys et al. 2013; Kang

et al. 2001; Moro et al. 2004; Velez-Lago et al. 2013) however in some instances the worsening of bradykinesia was reported (Huys et al. 2013). One case study found significant improvement of reflective and voluntary saccades, which are known to be impaired in HD patients (Fawcett et al. 2005). The majority of DBS case reports in HD, although limited to a small number of individual cases, suggest that it may be a treatment choice for drug-resistant chorea in HD (Huys et al. 2013).

DBS offers an ideal opportunity for translational experimental medicine approaches that are recognized to be critical to CNS drug discovery. Neurophysiological recordings before and after DBS can generate invaluable data about the pathophysiology of basal ganglia circuitry in HD, which can then be applied to engineer improved HD animal models with better mechanistic validity essential for preclinical drug discovery. Indeed, recording from GPi neurons in patients undergoing DBS revealed a surprisingly similar firing rate in HD and Parkinson's disease, but different firing patterns (Tang et al. 2005). CHDI is poised to generate such experimental medicine data and apply that to the development of novel therapeutics.

4.5 Modulation of Phosphodiesterases

Phosphodiesterases (PDEs) catalyze the degradation of cyclic nucleotides, and their inhibition leads to sustained signaling mediated by increased levels of cAMP, cGMP, or both. Intracellular cyclic nucleotide signaling plays a fundamental role in synaptic transmission, plasticity, and gene regulation (Hebb and Robertson 2007; Jeon et al. 2005; Rose et al. 2005). Specifically, signaling pathways downstream of cAMP elevation are deregulated in HD animal models and in HD patient postmortem samples (Ahn et al. 1997; DeMarch et al. 2008; Giampà et al. 2006, 2009; Gines 2003; Giralt et al. 2011; Kleiman et al. 2010; Obrietan 2004; Sathasivam et al. 2010; Sugars 2003). Administration of rolipram (a PDE4 selective inhibitor) and TP-10 (a selective PDE10 inhibitor) to HD mouse models were reported to delay disease progression (Demarch et al. 2007; Giampà et al. 2009; Giampà et al. 2009; Kleiman et al. 2010). PDE4 is cAMP-specific, whereas PDE10 modulates signaling by both cAMP and cGMP (Chandrasekaran et al. 2008; Iona et al. 1998; Handa et al. 2008; Jeon et al. 2005). Interestingly, PDE10 is one of the earliest and most significantly differentially downregulated transcripts in most HD animal models and in HD patient postmortem samples (Kleiman et al. 2010; Kotera 2003; Threlfell et al. 2008; Seeger 2003; Sotty et al. 2009). CHDI and collaborators have monitored expression of PDE10 in HD patients through the use of a specific PDE10 PET tracer, confirming the findings identified in HD rodent models (unpublished observations). As PDE10 is expressed in MSNs of the striatum, it is possible that loss of PDE10 expression is a compensatory mechanism downstream of synaptic alterations in basal ganglia circuitry (Kotera 2003; Seeger 2003).

In the basal ganglia, cAMP modulation via PDE4 and PDE10 inhibition plays fundamental roles in the modulation of DARPP32 phosphorylation (Nishi et al. 2005). The role of cGMP is less well understood, but recent work suggests that both cAMP and cGMP pathways play central roles in basal ganglia physiology and in corticostriatal connectivity (Kleiman et al. 2011; Rodefer et al. 2012; Schmidt et al. 2008; Sotty et al. 2009; Threlfell et al. 2009). In addition, the elevation of cAMP is required for memory processes in other systems affected in HD models (Bureau et al. 2006; Houslay et al. 2005; Kuroiwa et al. 2011; Li et al. 2011; Rodefer et al. 2012; Rose et al. 2005; Rutten et al. 2007, 2008; Spina 2008; Zhang et al. 2008). Studies conducted in rodent, NHPs, and humans with rolipram and other nonselective PDE4 inhibitors have highlighted the pro-cognitive effects in a variety of tasks involving both the hippocampal and the corticostriatal systems (Rose et al. 2005; Rodefer et al. 2012; Rutten et al. 2007, 2008) Rolipram administration leads to preclinical efficacy in rodents in the object recognition (Rutten et al. 2006), water maze (Nagakura et al. 2002), and passive avoidance tasks (Imanishi et al. 1997), and in an object retrieval (OR) task in NHPs (Rutten et al. 2008). Clinical studies using rolipram have investigated its potential utility to treat affective disorders and cognitive impairment, although its clinical development was halted due to adverse side effects (Bertolino et al. 1988; Hebenstreit et al. 1989; Spina 2008). The narrow therapeutic index (TI) of rolipram led to the development of selective subtype PDE4D and PDE4B orthosteric inhibitors (Bruno et al. 2011; Burgin et al. 2010; Huang et al. 2007; Kobayashi et al. 2011; Mitchell et al. 2010; Naganuma et al. 2009; Wang et al. 2007), and of negative allosteric modulators (NAMs) of PDE4D (Burgin et al. 2010; Robichaud et al. 1999, 2002). We have tested two of these PDE4D NAMs and confirmed their pro-cognitive activity in the OR task using young female *Cynomolgus* macaques (unpublished observations). We consider that molecules that elicit pro-cognitive effects in NHPs in tasks that reflect corticostriatal system modulation should be investigated in HD studies. The development of these PDE4D NAMs for HD will require the incorporation of methodologies to monitor dose-dependent effects in the circuitry thought to underlie HD symptoms.

Based on studies showing that various PDE inhibitors have some benefit in R6/2 phenotypes, we synthesized or procured from industrial collaborators a set of brain-penetrant, selective PDE inhibitors for use as proof-of-concept molecules for the treatment of HD. Our criteria for selection were selectivity and pharmacokinetic (PK) properties suitable for long-term studies in HD rodent models. We focused on the following enzymes; PDE1, -2, -5, -9, and -10. The evaluation of a PDE1 selective inhibitor in rodents showed that this enzyme does not significantly contribute to disease progression in R6.2 mice (unpublished observations). A PDE2 selective inhibitor was shown to improve acquisition and consolidation during a novel object recognition task (NOR), in social recognition paradigms (Boess et al. 2004) and in MK-801 impaired animals (Boess et al. 2004; Rutten et al. 2007). Our work with selective PDE inhibitors against PDE2, -9, and -10 obtained from Pfizer showed that inhibition of these enzymes, presumably via a modulation of cGMP levels intracellularly, can rescue the synaptic deficits

characterized in HD mouse models (Andre et al. 2010; Heikkinen et al. 2012) either in acute in vitro studies using corticostriatal and hippocampal slices, or after subchronic dosing studies, highlighting their potential for the treatment of HD (data unpublished). In addition, PDE10 inhibition rescues the alterations observed in subthalamic neuron (STN) firing in vivo, suggesting that the modulation of PDE10 activity can profoundly affect the circuitry in ways that are consistent with a potential beneficial clinical effect. We, in collaboration with Pfizer, are continuing to explore the preclinical and clinical efficacy of PDE9 and -10 inhibitors. The first Phase II safety and tolerability study using MP-10 will take place in the near future (clinicaltrials.gov identifier NCT01806896). Omeros has also reported in a press release of their development of another PDE10 inhibitor to treat HD. Additional testing of PDE9 inhibition, in the context of HD rodent models and in NHP cognition models, is still underway.

4.6 Modulation of BDNF/TrkB Signaling

The BDNF neurotrophin pathway has been extensively associated with HD pathology since it was shown that TrkB (the tyrosine kinase receptor for BDNF) is required for proper maturation and homeostasis of the striatum in rodents, and that BDNF levels are decreased in HD rodent models. Most notable is the pleiotropic role of BDNF signaling during multiple facets of MSN differentiation, function, and survival and, in particular, with the indirect-pathway MSNs expressing D2 dopamine receptors and enkephalin (a population thought to be differentially affected in HD). Significantly, the effect of BDNF signaling on synaptic plasticity and axonal transport in MSNs reflects a critical role for this pathway in HD pathophysiology. Therefore, the modulation of this pathway is a critical component of the therapeutic strategy for HD (Arenas et al. 1996; Arregui et al. 2011; Besusso et al. 2013; Brito et al. 2013; Buckley et al. 2010; Canals et al. 2004; Conforti 2013; Conforti et al. 2013; Giampa et al. 2013; Giralt et al. 2011; Goggi et al. 2002; Ivkovic and Ehrlich 1999; Jiang et al. 2013; Jiao et al. 2011; Kells et al. 2004; Liot et al. 2013; Martire et al. 2013; Massa et al. 2010; Soldati et al. 2011; Xie et al. 2010; Zala et al. 2008; Zuccato et al. 2011).

From a therapeutic perspective, activation of TrkB selectively in the basal ganglia might be disease modifying, based on studies in HD rodent models. The key question is how best to modulate TrkB signaling while minimizing potential adverse effects associated with BDNF signaling through either the p75 receptor or in regions outside the basal ganglia associated with weight loss (hypothalamus), seizure activity (hippocampus), and/or pain (amygdala) (Perreault et al. 2013; Tsao et al. 2008; Vanevski and Xu 2013; Waterhouse and Xu 2013). It is well established that TrkB activation in the hypothalamus and brain stem leads to significant weight alterations, which might be an unacceptable side effect in HD patients, in whom weight loss is a significant comorbidity. However, more recent evidence suggests that the weight alteration effects of peripheral TrkB activation with

monoclonal agonistic antibodies in rodents and NHPs is opposite, leading to weight gain (orexigenic) rather than loss (anorexigenic) in NHPs, diminishing this concern (Perreault et al. 2013; Tsao et al. 2008). However, the effects of BDNF administration centrally versus peripherally in NHPs and its opposing results in body weight regulation highlights that careful biodistribution and toxicity studies need to be conducted to understand whether direct administration of an antibody agonist to TrkB in the striatum might be a viable therapeutic strategy for HD (Perreault et al. 2013; Tsao et al. 2008; Vanevski and Xu 2013; Waterhouse and Xu 2013).

Although there have been several reports of small molecule 'mimetics' of BDNF and cyclic peptides presumed to activate TrkB receptors, we have not been able to reproduce any of these findings utilizing a comprehensive set of cellular assays, including TrkB dimerization, phosphorylation, and signaling via ERK and pI3 K pathways, calling into question whether the reported effects truly reflect a direct modulation of this signaling pathway (Fletcher and Hughes 2009; Jiang et al. 2013; Marongiu et al. 2013; Massa et al. 2010; O'Leary and Hughes 2003). We have also unsuccessfully attempted to identify novel TrkB-interacting small molecules through computational modeling approaches. CHDI has now abandoned the pursuit of small-molecule TrkB activators as a therapeutic strategy for HD, and have redirected our efforts to activating TrkB via monoclonal antibodies, or via central delivery of BDNF itself utilizing AAV. Currently, these efforts are in preclinical rodent testing stages.

Other potential strategies of specific relevance to HD relate to the role of REST/NRSF in linking mutant HTT expression to the loss of BDNF expression observed in the forebrain (Buckley et al. 2010; Conforti et al. 2013; Xie et al. 2010). These effects appear to be directly connected to mHTT function, and are recapitulated in cellular systems. The molecular modulation of REST expression is an intriguing avenue therapeutically, but given the complexity of conducting gene delivery clinical trials additional in vivo work is required to follow up on the exciting initial findings in rodent models, and extending these studies to understand potential adverse effects of constitutively repressing REST expression in the adult forebrain (Buckley et al. 2010; Conforti 2013; Conforti et al. 2013).

A significant challenge to clinically investigating these approaches is the lack of biomarkers of activation of the TrkB signaling cascade in the basal ganglia. These might include the modulation of enkephalin levels in CSF, or the modulation of cannabinoid or GABA signaling, which have been shown in rodents to be responsive to BDNF levels (Arenas et al. 1996; De Chiara et al. 2010; Goggi et al. 2002; Ivkovic and Ehrlich 1999; Jiao et al. 2011). Of note, one of the primary roles for BDNF in development is the specification of GABAergic neuronal fates, perhaps linking the neurodevelopmental role for BDNF to the specific degeneration of GABAergic neurons of the striatum in HD. Nonetheless, our prediction is that activation of TrkB signaling in the striatum will be reflected in changes in extracellular GABA or cannabinoid levels in the striatum, opening the way for noninvasive measurements in a clinical context.

5 Small Molecules Targeting Cellular Energetic Mechanisms

Energetic alterations are commonly described in every neurodegenerative condition, including HD. Deficits in glycolysis or the electron transport chain can lead to profound mental retardation, and striatal degeneration is well documented in many inborn errors of metabolic disorders. In HD animal models and in premanifest and manifest humans there appear to be signs of metabolic and energetic disturbances, both in muscle and brain tissues (Gellerich et al. 2008; Heikkinen et al. 2012; Mochel and Haller 2011; Mochel et al. 2010, 2012; Reynolds et al. 2008; Tabrizi et al. 2003; van den Bogaard et al. 2011). The investigation of the exact deficits and the molecular links between mHTT function and these energetic deficiencies needs further investigation. However, results deriving from allelic series of mice and cells expressing various expansions of the polyQ stretch, as well as from cells lacking HTT function, suggest that the link between HTT function and energetic mechanisms might be more direct than previously thought (Gines et al. 2003; Seong et al. 2005). This molecular connection is unique among adult-onset neurodegenerative disorders, and places HD at the center of the therapeutic strategies directed toward a restoration of brain energy homeostasis.

Most of the characterization of energetic deficits has been in cells or tissues, processed to evaluate changes in adenine nucleotides, creatine, and phosphocreatine to assess the energy charge of the cell. In general, the findings obtained in HD patients and HD rodent models are not always consistent, although this is likely to be confounded by tissue measurements as opposed to the measurement of specific cell populations during disease progression. Glycolytic and mitochondrial energetic mechanisms in vulnerable neuronal populations, and in glia, have not been adequately analyzed. The interplay between glia and neurons from an energetics perspective in HD is all but unexplored. In humans, the most notable findings are alterations in creatine/phosphocreatine levels quantified by magnetic resonance spectroscopy (MRS), although these studies involved few patients and did not follow them longitudinally (Reynolds et al. 2008; van den Bogaard et al. 2011). Studies in muscle in gene-positive carriers have shown that there are detectable changes in premanifest subjects that can be measured after challenge paradigms such as during exercise tests (unpublished).

Evidence of abnormal energy utilization has come from monitoring energetic endpoints and lactate production in muscle during an exercise test in 25 HD patients. This small study showed elevated lactate levels and lower anaerobic threshold in HD subjects, for manifest and premanifest phases of disease (Ciammola et al. 2011). Other studies have revealed variable effects in brain lactate levels, and altered glutamate/glutamine ratios in MRS studies (Jenkins et al. 1998; Mochel et al. 2010; Reynolds et al. 2008; Tabrizi et al. 2003; van den Bogaard et al. 2011). Creatine kinase (CK) deficiency has been linked to HD, and supplementation of brain isoforms of CK shows positive effects in rodent models of HD (Kim et al. 2010; Lin et al. 2011; Zhang et al. 2011). Typically, the studies are

cross-sectional and with small sample sizes, making it difficult to rigorously evaluate whether the changes detected are robust, reproducible, and progressive during the clinical evolution of symptoms. Clearly, a more systematic effort needs to be conducted across multiple sites to definitively address the nature of the energetic alterations, both in brain regions and muscle of HD subjects. The usefulness of these measurements as disease progression biomarkers might be questionable based on existing data, but these can potentially be incorporated into clinical studies as pharmacodynamic biomarkers, which typically have not been included in the ongoing therapeutic trials (see below).

Due to the availability of well tolerated antioxidant molecules, several large HD clinical trials are underway to probe the hypothesis that elevated reactive oxygen species, or deficits in energy homeostasis, contribute to HD progression. The clinical studies have evaluated the effects of cysteamine (CYTE-I-HD study), creatine (CREST-E study), ethyl-EPA (TREND-HD study), and coenzyme Q10 (2CARE study; see Table 1). Most of these studies, in spite of conflicting findings from rodent studies (Borrell-Pages et al. 2006; Hickey et al. 2012; Menalled et al. 2010; Yang et al. 2009; Van Raamsdonk et al. 2005), have not yielded any strong evidence for efficacy in clinical trials. In Phase II, some positive results have been documented for the studies involving CoQ10, ethyl-EPA, and creatine, but these are likely underpowered. Other studies have been completed to assess tolerability and safety in HD (Dubinsky and Gray 2006; Hersch et al. 2006; Huntington Study Group TREND-HD 2008; Hyson et al. 2010; Puri et al. 2002, 2005; Tabrizi et al. 2003; Verbessem et al. 2003). Unfortunately, most clinical studies did not incorporate pharmacodynamic or energetic measures pre- and post-treatment, giving no definitive reason for the negative findings in pivotal trials. Therefore, the mechanisms purported to be modulated by these drugs remain untested. In an initial clinical study with creatine, levels of the metabolite 8-OH2dG, thought to arise from free reactive oxygen radical generation in the mitochondria, were shown to be decreased after administration of creatine (Hersch et al. 2006). However, recent evidence shows that 8-OH2dG levels do not correlate with HD progression, casting doubt on the usefulness of this biomarker to monitor energetic disturbances in HD (Borowsky et al. 2013).

In terms of novel potential therapeutics aimed at these mechanisms in preclinical stages, the pathways that stand out are the AMPK, PPARγ, and PGC1α pathways (Chaturvedi and Beal 2013; Chaturvedi et al. 2009; Jeong et al. 2012; Jiang et al. 2012; Jin et al. 2013; Johri et al. 2012; Mochel et al. 2012; Vlamings et al. 2012; Weydt et al. 2006). Several reports point to deficiencies in PGC1α as an underlying deficit which might explain the mitochondrial abnormalities reported in humans and rodent models of the disease (Chaturvedi et al. 2009; Weydt et al. 2006). As PGC1α itself is not amenable to direct pharmacological modulation other approaches have been explored to activate PGC1α transcriptional activity, through either the use of small-molecule agonists of the nuclear hormone receptor PPARγ, such as rosiglitazone (Jin et al. 2013), or the molecular modulation of other pathways which affect PGC1α levels or activity (via post-translational modifications), such as that observed through the AMPK or Sirt pathways

(Chaturvedi et al. 2009; Ho et al. 2010; Mochel et al. 2012; Nin et al. 2012; Weydt et al. 2006; Zhao et al. 2008). Brain-penetrant molecules are being tested in various HD models to understand how the specific modulation of mitochondrial number or health might affect disease endpoints. Finally, other approaches targeting energetic mechanisms are in early preclinical stages in HD research, including the antioxidant response system mediated by the Nrf2/Keap complex (Johnson et al. 2008). Considerably more effort needs to be placed in identifying suitable molecules that can be used as in vivo mechanistic tools, with a well understood pharmacological profile and established PK/PD correlations to facilitate querying these mechanisms in the brain and assess their relevance to HD progression.

6 Other Small Molecule Drug Discovery Programs

6.1 Selective Inhibitors of KMO

The kynurenine pathway is the major catabolic route for tryptophan metabolism, and a number of neuroactive molecules have been described, including kynurenic acid (KYNA), 3-hydroxy-anthranilic acid (3-HAA), 3-hydroxy-kynurenine (3-HK), and quinolinic acid (QUIN). Metabolites in the kynurenine pathway (KP) have been implicated in the pathophysiology of psychiatric and neurodegenerative disorders, including HD, and their modulation via selective drugs targeting key enzymes in this pathway is an area of active investigation (Schwarcz et al. 2012; Vecsei et al. 2013). The main findings implicating this pathway's relevance to a variety of CNS indications rest upon the following: metabolite levels are altered as compared to baseline levels; in the context of HD, the main finding is an elevation in 3-HK levels, although some reports of decreased KYNA levels also exist (Forrest et al. 2010; Guidetti et al. 2000, 2004, 2006; Mazarei et al. 2013; Sapko et al. 2006; Sathyasaikumar et al. 2010; Schwarcz et al. 2012; Vecsei et al. 2013). By inference, the elevation in 3-HK levels in brains of HD animal models, or in HD patient-derived fluids or tissues, implicates KMO activity as the culprit. Some kynurenines have been implicated in the modulation of nicotinic signaling (through a modulation of the α7 subunit of the nAchR), and in the modulation of NMDA or AMPA receptors by QUIN and KYNA (Alkondon et al. 2004, 2011; Konradsson-Geuken et al. 2010; Lopes et al. 2007; Pocivavsek et al. 2011; Stone and Darlington 2002), although some of these effects have been disputed (Mok et al. 2009; Vecsei et al. 2013) and the role of KYNA at physiological concentrations in the brain (low nM) remain uncertain. The physiology of this pathway necessitates carefully controlled studies with selective and potent molecules, rather than relying on exogenously administered kynurenines or genetic models that are typically poorly characterized. The role of kynurenines in the inflammatory response and the intersection between inflammation and neurotransmitter

pathways remains an area of intense interest. The interplay between neuroactive kynurenines and inflammatory signaling events in the brain has not been adequately investigated, yet the enzyme that triggers the kynurenine cascade, indoleamine 2,3-dioxygenase (Ido), is a critical component of neuroinflammation in response to viral or bacterial pathogens (Haroon et al. 2012).

In genetic screens in *Drosophila* and yeast, KMO seems to be necessary for the toxic effects of exogenous, overexpressed, mutant human HTT-driven toxicity (Campesan et al. 2011; Forrest et al. 2010; Guidetti et al. 2000, 2004; Sapko et al. 2006; Sathyasaikumar et al. 2010; Schwarcz et al. 2012; Vecsei et al. 2013; Zwilling et al. 2011). The effects of the loss of function of KMO in these systems might be driven through the modulation of 3-HK levels, and its effects as a reactive oxygen species generator (Okuda et al. 1998). The relevance of this finding to the human disease is unclear, although the elevation of 3-HK levels as a potential pathogenic mechanism seems to be the most consistent finding in all of these studies and in human disease (Christofides et al. 2006; Forrest et al. 2010; Guidetti et al. 2004). In spite of several studies conducted to monitor kynurenine levels in HD during disease progression or in presymptomatic individuals, the sample sizes reported in these studies are low. In addition, the reliance of postmortem tissue sampling as a mechanism to study the involvement of this pathway lacks adequate power to reach any meaningful conclusion. A definitive answer about the timing, magnitude, and nature of these presumed alterations, and their relationship to an immunological alteration in these subjects, remains to be confirmed. Few well-controlled studies have been conducted in which CSF levels of kynurenines are measured longitudinally, a necessary step to understand whether this pathway is correlated with disease progression, inflammation, or both, in HD.

Overall, the existing evidence suggests that KMO inhibition might be beneficial to HD, although the mechanisms by which KMO inhibition could slow disease progression are unknown. In an important study, Zwilling et al. reported that KMO inhibition in rodent models of HD can ameliorate disease progression via a presumed KMO inhibitor, the JM6 molecule (Zwilling et al. 2011). However, our studies have shown that JM6 is not a potent KMO inhibitor (Beconi et al. 2012), and have been unable to replicate these findings with our potent and selective KMO inhibitors, bringing into question the main findings of this paper. We have developed a novel, selective KMO inhibitor which can modulate the pathway effectively in a dose-dependent manner, leading to an elevation of KYNA in brain, as quantified by microdialysis and CSF measurements, both in rodents and NHPs (unpublished data). Overall, the therapeutic potential of this mechanism awaits clinical studies after it is demonstrated that systemic KMO inhibition leads to a normalization of kynurenine changes (if indeed they exist in HD), studies that are planned. Given the multiple modes of action downstream of KMO inhibition and the implications of kynurenine metabolite levels in several domains of relevance to HD (Haroon et al. 2012; Stone and Darlington 2002; Vecsei et al. 2013), a clinical study is the most expeditious way to test these hypotheses in humans. For example, is the elevation of KYNA necessary for synaptic or neuroprotective effects, is the decrease in neurotoxic metabolites (3-HK and QUIN), the modulatory effects in

the immune system, or all of the above, required for KMO inhibition to be an efficacious therapeutic for HD?

6.2 Selective Modulators of Lysine Deacetylases

The HDACs (histone deacetylases; herein referred to as lysine deacetylases) are composed of 11 enzymes, Class I (1–3 and 8), IIa (4, 5, 7, and 9) and IIb (6, 10), Class III (sirtuins 1–6), and Class IV (HDAC 11) (Choudhary et al. 2009). Nonselective HDAC inhibitors such as SAHA, sodium butyrate, and TSA have been reported to ameliorate disease phenotypes in HD models. Subsequent work has identified a subset of HDACs whose modulation modifies disease progression in a variety of HD models, such as HDAC-2, -3, and -4. (Hockly et al. 2003; Jia et al. 2012a, b; McFarland et al. 2012; Mielcarek et al. 2011; Quinti et al. 2010; Sadri-Vakili et al. 2007; Steffan et al. 2001; Thomas et al. 2008). Both SAHA and TSA are broad spectrum inhibitors of Class I and are weak Class IIa and III inhibitors. Because of the initial encouraging findings of nonselective Class I HDAC inhibitors in HD models, a large effort was undertaken to identify the HDAC subtype responsible for the beneficial effects of these inhibitors. Although SAHA and TSA administration showed promising results in HD models, their therapeutic index is low, and significant side effects were observed during dose-escalation studies which limited a more in-depth investigation of their therapeutic potential. In addition, the pharmaceutical properties of these molecules in terms of their PK/PD profiles are suboptimal for a chronic condition such as HD. Because of this, we collaborated with Gillian Bates group at King's College London to systematically assess the genetic contribution of various HDAC genes to the pathology of the R6/2 model of HD.

They generated a large number of genetic crosses between the R6/2 mice and single gene deletion strains for the various Class I and Class II HDACs, either as complete homozygote deletion animals or as heterozygous crosses since several of the HDAC genes induce embryonic lethality in mice when completely deleted. Bates' group has shown that the only HDAC gene deletion that substantially delays R6/2 phenotypes is a partial loss of HDAC (Benn et al. 2009; Bobrowska et al. 2011; Moumne et al. 2012) hed (Mielcarek et al., PLOS Biology, in press). HDAC4 heterozygote animals crossed with R6/2 mice demonstrated a significant delay in progression in a variety of endpoints, including survival, locomotor endpoints, synaptic dysfunction in hippocampal and corticostriatal acute slice assays, HTT aggregation, and a rescue of a subset of cortical transcriptional alterations (such as in BDNF transcription) frequently observed in most HD models. The mechanism by which loss of HDAC expression leads to these impressive effects on disease progression in the R6/2 model is unclear, but it might relate to the direct interaction between HDAC4 and HTT, as shown by co-immunoprecipitation studies using brain and cellular extracts. Support for the role of HDAC4 in mediating HTT toxicity has also been obtained in cellular systems of HD by other investigators (Jovicic et al. 2013). The recent finding that chronic

SAHA administration leads to changes in HDAC2 and -4 protein levels, and the similarities in some of the beneficial effects observed with SAHA and in the genetic loss of HDAC4 expression, suggest that one of the mechanisms by which SAHA can modify disease progression might be through a partial loss of function of HDAC4.

The case for the involvement of Class I HDACs in HD pathogenesis is strong based on the inhibitor studies described above, although the side effect profiles of these compounds and their broad effect on transcriptional (histone-modification driven) alterations make them difficult to develop for chronic indications. However, recent evidence implicate HDAC3 in HD pathophysiology, although Bates' group did not find supporting evidence in a partial loss of HDAC3 expression in an R6/2 context. In addition, some of the claims made using the HDAC-4b compound (that has higher affinity for HDAC3 and -1) were called into question since we did not replicate the findings in HD models dosed with this compound, which has poor PK properties for brain modulation of HDAC biology (Beconi et al. 2012; Jia et al. 2012; Moumne et al. 2012; Thomas et al. 2008). Nonetheless, the notion remains that a Class I HDAC modulator either optimized for isotype selectivity (HDAC3) or having a better safety profile might be useful in HD. In spite of the disagreements about the mechanism of action in vivo of the HDAC-4b compound, or its efficacy in rodent models of HD, HDAC3 as an enzyme remains of interest for HD. In the context of HDAC4 biology (and more broadly in Class II HDAC biology), the interaction between HDAC4 and the HDAC3-N-CoR and SMRT corepressor complexes (Guenther et al. 2001; Wen et al. 2000) might indicate that both of these proteins form part of a complex with strong functional interaction with HTT itself, or HTT-dependent mechanisms that correlate with disease pathogenesis. The biggest hurdle for drug development in this area (see below) remains a deeper understanding of how this complex intersects with HTT biology so that suitable biological endpoints (other than histone acetylation changes) can be incorporated into the drug discovery process.

Based on the findings of the Bates group now substantiated in three different genetic models of HD (unpublished findings and Mielcarek et al., in press), we developed potent, selective Class IIa HDAC inhibitors with suitable PK properties for chronic dosing in HD models, which are currently being tested in various rodent HD models to determine whether inhibition (or binding in the catalytic active site) can recapitulate the genetic phenotypes. Our medicinal chemistry program focused on the development of active site inhibition for Class II enzymes. Our lead molecules display 1,000-fold selectivity over Class I and III enzymes, and a subset of the molecules can disrupt an HDAC4-3 complex in a cellular context. These inhibitors bind to the active site of Class II HDACs as demonstrated by crystallography studies (manuscript in revision). The big question is whether a catalytic inhibitor (with the ability to disrupt protein–protein interactions) can recapitulate the effects observed in the genetic studies as reported by the Bates group. In addition to these traditional medicinal chemistry efforts, we are exploring other molecular approaches as a potential therapeutic to target HDAC4 expression.

Class III lysine deacetylases of the sirtuin family have received considerable interest given their role in aging, metabolism, and DNA damage mechanisms (Hall et al. 2013; Sebastian et al. 2012). Sirtuins are NAD+ dependent lysine deacetylases, and it is the requirement for NAD+ binding for catalysis that explains their involvement in energy-sensing mechanisms, including the DNA damage response and energetic mechanisms. In the context of HD, the enzymes SIRT1 and -2 have been implicated in various biochemical pathways thought to be important for disease progression; these include the p53 signaling response in the context of DNA damage and apoptosis, and the mitochondrial deficits attributed to the loss of PGC1α (Jeong et al. 2012; Jiang et al. 2012; Ho et al. 2010; Hall et al. 2013; Sebastian et al. 2012; Shin et al. 2013) signaling. However, the relative involvement of SIRT1 and -2 in pathogenesis is controversial (Jeong et al. 2012; Jiang et al. 2012; Gertz et al. 2013; Ho et al. 2010; Hall et al. 2013; La Spada 2012; Luthi-Carter et al. 2010; Sebastian et al. 2012; Shin et al. 2013). For SIRT1 modulation, both inhibition and activation have been reported to affect disease phenotypes; in the case of Sirt2, findings in genetic deletion studies contrast with the results obtained with small-molecule inhibitors of SIRT1 and 2 (Bobrowska et al. 2012; Jeong et al. 2012; Jiang et al. 2012; Luthi-Carter et al. 2010).

Selisistat (EX-527), a Sirtuin1,2 inhibitor developed by Elixir Pharmaceuticals and Siena Biotech, entered clinical trials for HD in 2010, on the hypothesis that SIRT1 inhibition modulates HTT levels. The outcome of this study has not been revealed in the context of a biological or pharmacodynamic effect in HD patients, but results are expected soon. The development of 'sirtuin activators' remains an area of active investigation, and one fraught with controversy, given the artifactual findings with resveratrol and several Sirtris molecules (Beher et al. 2009; Dai et al. 2010; La Spada 2012). The controversy stems from the artifactual results of direct interaction between these small molecules and SIRT1 activation. We have evaluated most available Sirtris molecules and resveratrol and found no evidence for direct activation of SIRT1, as have others (Beher et al. 2009). Nonetheless, some of these molecules are making their way through medicinal chemistry efforts, and the jury is still out regarding whether they will show any efficacy in HD, and whether a chemical approach can indeed modulate SIRT1 activation directly (La Spada 2012). We attempted to identify small molecule direct interactors of Sirt1 with an activating potential and were unsuccessful. Other therapeutic strategies to modulate SIRT1 activity include the inhibition of Dbc1-SIRT1 interaction, or the modulation of upstream modulators of SIRT1 modulating proteins, such as AROS or various enzymes associated with the regulation of NAD+ levels or SIRT1 activity via post-translational modifications (Hubbard et al. 2013; Nin et al. 2012; Raynes et al. 2013; Zhao et al. 2008).

6.3 Future Approaches for Disease Modification Centered on HTT Post-Translational Modifications

HTT has hundreds of binding partners (Miller et al. 2011; Shirasaki et al. 2012) and displays a multitude of post-translational modifications (PTMs) including phosphorylation, proteolysis, ubiquitination, sumoylation, acetylation, and palmitoylation (Ehrnhoefer et al. 2011). Many of these PTMs are thought to affect HTT localization, its interaction with various binding partners, its function, and its propensity to aggregate (Aiken et al. 2009; Atwal et al. 2011; Ehrnhoefer et al. 2011; Hogel et al. 2012; Kegel et al. 2009; Mishra et al. 2012; Trevino et al. 2012). The interplay between these PTMs and HTT function are unclear but several recent reports have demonstrated that modification of several residues thought to be modified in mutant versus wild type HTT can have profound effects on mHTT toxicity, aggregation, clearance, or subcellular localization (Ehrnhoefer et al. 2011; Jeong et al. 2009; Khoshnan and Patterson 2011; Zala et al. 2008).

Analysis of the correlation between polyQ length with the 'motor age of onset' suggests that it is not the only determinant of pathogenesis and disease progression, and that other factors contribute to the variance in disease onset. In various HD rodent models with different polyQ lengths, disease severity, and progression seems to be modulated by HTT expression levels. There is also evidence of other modifier loci and recent studies have indicated that HTT oligomerization, inclusion formation, and toxicity are influenced by regions outside the polyQ region (Tang et al. 2011; Wang and Lashuel 2013), suggesting that modulation of PTMs affecting HTT function, aggregation, or localization can act to overcome the pathogenic mechanisms triggered by expanded polyQ. Mutations, deletions, and PTMs within the three flanking sequences of the polyQ domain in HTT exon1 (N-terminal 17AA (Nt17), polyQ, and proline rich domain (PRD) strongly affect cellular processes, propensity to aggregate and toxicity of mHTT in HD transgenic models (flies and in mice) (Tam et al. 2009; Thakur et al. 2009).

These findings suggest that it is theoretically possible to modify mHTT toxicity via upstream enzymes or signaling pathways responsible for the PTMs of such critical amino acids or structural motifs within HTT. However, the development of therapeutic strategies targeting the critical enzymes (or pathways) responsible for these PTMs of HTT is challenging due to lack of sufficient clarity regarding the enzymes or mechanisms that are necessary and sufficient in vivo for these modifications. An initial screen detailing the identification of kinases modifying two such residues (pSer13/pSer16) has recently been reported (Atwal et al. 2011). To better understand the effect of a specific PTM on mHTT toxicity, CHDI in collaboration with various academic researchers have developed a plan to identify these modifications systematically, and to investigate upstream mechanisms that might be pharmacologically tractable. CHDI is currently developing antibodies specific for the best validated PTMs, a necessary step in conducting molecular and chemical screens via high content imaging (HCS) or other antibody-based technologies to identify mechanisms responsible for these modifications. We predict

that in the next few years the role of some of these modifications in mediating, or ameliorating, HTT-driven toxicity, will be elucidated, enabling a novel approach to slow disease progression employing more conventional approaches for drug development.

References

Ahn HS, Bercovici A, Boykow G et al (1997) Potent tetracyclic guanine inhibitors of PDE1 and PDE5 cyclic guanosine monophosphate phosphodiesterases with oral antihypertensive activity. J Med Chem 40:2196–2210

Aiken CT, Steffan JS, Guerrero CM et al (2009) Phosphorylation of threonine 3: implications for Huntingtin aggregation and neurotoxicity. J Biol Chem 284:29427–29436

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375

Alkondon M, Pereira EF, Yu P et al (2004) Targeted deletion of the kynurenine aminotransferase ii gene reveals a critical role of endogenous kynurenic acid in the regulation of synaptic transmission via alpha7 nicotinic receptors in the hippocampus. J Neurosci 24:4635–4648

Alkondon M, Pereira EF, Albuquerque EX (2011) Endogenous activation of nAChRs and NMDA receptors contributes to the excitability of CA1 stratum radiatum interneurons in rat hippocampal slices: effects of kynurenic acid. Biochem Pharmacol 82:842–851

Allen KL, Waldvogel HJ, Glass M et al (2009) Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. J Chem Neuroanat 37:266–281

Andrade MA, Bork P (1995) HEAT repeats in the Huntington's disease protein. Nat Genet 11:115–116

Andre VM, Cepeda C, Levine MS (2010) Dopamine and glutamate in Huntington's disease: a balancing act. CNS Neurosci Ther 16:163–178

Arenas E, Akerud P, Wong V et al (1996) Effects of BDNF and NT-4/5 on striatonigral neuropeptides or nigral GABA neurons in vivo. Eur J Neurosci 8:1707–1717

Arregui L, Benitez JA, Razgado LF et al (2011) Adenoviral astrocyte-specific expression of BDNF in the striata of mice transgenic for Huntington's disease delays the onset of the motor phenotype. Cell Mol Neurobiol 31:1229–1243

Atwal RS, Desmond CR, Caron N et al (2011) Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol 7:453–460

Baldo B, Paganetti P, Grueninger S et al (2012) TR-FRET-based duplex immunoassay reveals an inverse correlation of soluble and aggregated mutant huntingtin in huntington's disease. Chem Biol 19:264–275

Bari M, Battista N, Valenza M et al (2013) In vitro and in vivo models of Huntington's disease show alterations in the endocannabinoid system. FEBS J 280:3376–3388

Bartus RT, Baumann TL, Brown L et al (2013) Advancing neurotrophic factors as treatments for age-related neurodegenerative diseases: developing and demonstrating "clinical proof-of-concept" for AAV-neurturin (CERE-120) in Parkinson's disease. Neurobiol Aging 34:35–61

Becher MW, Kotzuk JA, Sharp AH et al (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol Dis 4:387–397

Beconi MG, Yates D, Lyons K et al (2012a) Metabolism and pharmacokinetics of JM6 in mice: JM6 is not a prodrug for Ro-61-8048. Drug Metab Dispos 40:2297–2306

Beconi M, Aziz O, Matthews K et al (2012b) Oral administration of the pimelic diphenylamide HDAC inhibitor HDACi 4b is unsuitable for chronic inhibition of HDAC activity in the CNS in vivo. PLoS One 7:e44498

Beher D, Wu J, Cumine S et al (2009) Resveratrol is not a direct activator of SIRT1 enzyme activity. Chem Biol Drug Des 74:619–624
Benn CL, Butler R, Mariner L et al (2009) Genetic knock-down of HDAC7 does not ameliorate disease pathogenesis in the R6/2 mouse model of Huntington's disease. PLoS One 4:e5747
Bertolino A, Crippa D, di Dio S et al (1988) Rolipram versus imipramine in inpatients with major, minor or atypical depressive disorder: a double-blind double-dummy study aimed at testing a novel therapeutic approach. Int Clin Psychopharmacol 3:245–253
Besusso D, Geibel M, Kramer D et al (2013) BDNF-TrkB signaling in striatopallidal neurons controls inhibition of locomotor behavior. Nat Commun 4:2031
Biglan KM, Ross CA, Langbehn DR et al (2009) Motor abnormalities in premanifest persons with Huntington's disease: the PREDICT-HD study. Mov Disord 24:1763–1772
Blazquez C, Chiarlone A, Sagredo O et al (2011) Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. Brain 134:119–136
Bobrowska A, Paganetti P, Matthias P et al (2011) Hdac6 knock-out increases tubulin acetylation but does not modify disease progression in the R6/2 mouse model of Huntington's disease. PLoS One 6:e20696
Bobrowska A, Donmez G, Weiss A et al (2012) SIRT2 ablation has no effect on tubulin acetylation in brain, cholesterol biosynthesis or the progression of Huntington's disease phenotypes in vivo. PLoS One 7:e34805
Boess FG, Hendrix M, van der Staay FJ et al (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. Neuropharmacology 47:1081–1092
Borowsky B, Warner J, Leavitt BR et al (2013) 8OHdG is not a biomarker for Huntington disease state or progression. Neurology 80:1934–1941
Borrell-Pages M, Canals JM, Cordelieres FP et al (2006) Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via HSJ1b and transglutaminase. J Clin Invest 116:1410–1424
Brandt J, Folstein SE, Wong DF et al (1990) D2 receptors in Huntington's disease: positron emission tomography findings and clinical correlates. J Neuropsychiatry Clin Neurosci 2:20–27
Brito V, Puigdellivol M, Giralt A et al (2013) Imbalance of p75(NTR)/TrkB protein expression in Huntington's disease: implication for neuroprotective therapies. Cell Death Dis 4:e595
Bruno O, Fedele E, Prickaerts J et al (2011) GEBR-7b, a novel PDE4D selective inhibitor that improves memory in rodents at non-emetic doses. Br J Pharmacol 164:2054–2063
Buckley NJ, Johnson R, Zuccato C et al (2010) The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. Neurobiol Dis 39:28–39
Bureau Y, Handa M, Zhu Y et al (2006) Neuroanatomical and pharmacological assessment of Fos expression induced in the rat brain by the phosphodiesterase-4 inhibitor 6-(4-pyridylmethyl)-8-(3-nitrophenyl) quinoline. Neuropharmacology 51:974–985
Burgin AB, Magnusson OT, Singh J et al (2010) Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. Nat Biotechnol 28:63–70
Cachope R (2012) Functional diversity on synaptic plasticity mediated by endocannabinoids. Philos Trans R Soc Lond B Biol Sci 367:3242–3253
Campesan S, Green EW, Breda C et al (2011) The kynurenine pathway modulates neurodegeneration in a Drosophila model of Huntington's disease. Curr Biol 21:961–966
Canals JM, Pineda JR, Torres-Peraza JF et al (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 24:7727–7739
Cattaneo E, Zuccato C, Tartari M (2005) Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci 6:919–930
Caviston JP, Holzbaur EL (2009) Huntingtin as an essential integrator of intracellular vesicular trafficking. Trends Cell Biol 19:147–155
Cepeda C, Galvan L, Holley SM et al (2013) Multiple sources of striatal inhibition are differentially affected in Huntington's disease mouse models. J Neurosci 33:7393–7406

Chandrasekaran A, Toh KY, Low SH et al (2008) Identification and characterization of novel mouse PDE4D isoforms: Molecular cloning, subcellular distribution and detection of isoform-specific intracellular localization signals. Cell Signal 20:139–153
Chaturvedi RK, Beal MF (2013) Mitochondria targeted therapeutic approaches in Parkinson's and Huntington's diseases. Mol Cell Neurosci 55:101–114
Chaturvedi RK, Adhihetty P, Shukla S et al (2009) Impaired PGC-1alpha function in muscle in Huntington's disease. Hum Mol Genet 18:3048–3065
Chen JJ, Ondo WG, Dashtipour K et al (2012) Tetrabenazine for the treatment of hyperkinetic movement disorders: a review of the literature. Clin Ther 34:1487–1504
Chiodi V, Uchigashima M, Beggiato S et al (2012) Unbalance of CB1 receptors expressed in GABAergic and glutamatergic neurons in a transgenic mouse model of Huntington's disease. Neurobiol Dis 45:983–991
Chou SY, Lee YC, Chen HM et al (2005) CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. J Neurochem 93:310–320
Choudhary C, Kumar C, Gnad F et al (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325:834–840
Christofides J, Bridel M, Egerton M et al (2006) Blood 5-hydroxytryptamine, 5-hydroxyindole-acetic acid and melatonin levels in patients with either Huntington's disease or chronic brain injury. J Neurochem 97:1078–1088
Ciammola A, Sassone J, Sciacco M et al (2011) Low anaerobic threshold and increased skeletal muscle lactate production in subjects with Huntington's disease. Mov Disord 26:130–137
Conforti P (2013) Mas Monteys, A.; Zuccato, C., et al. In vivo delivery of DN:REST improves transcriptional changes of REST-regulated genes in HD mice. Gene Ther 20:678–685
Conforti P, Zuccato C, Gaudenzi G et al (2013) Binding of the repressor complex REST-mSIN3b by small molecules restores neuronal gene transcription in Huntington's disease models. J Neurochem 127(1):22–35
Crook ZR, Housman D (2011) Huntington's disease: can mice lead the way to treatment? Neuron 69:423–435
Curtis A, Mitchell I, Patel S et al (2009) A pilot study using nabilone for symptomatic treatment in Huntington's disease. Mov Disord 24:2254–2259
Dai H, Kustigian L, Carney D et al (2010) SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator. J Biol Chem 285:32695–32703
De Chiara V, Angelucci F, Rossi S et al (2010) Brain-derived neurotrophic factor controls cannabinoid CB1 receptor function in the striatum. J Neurosci 30:8127–8137
De Yebenes JG, Landwehrmeyer B, Squitieri F et al (2011) Pridopidine for the treatment of motor function in patients with Huntington's disease (MermaiHD): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Neurol 10:1049–1057
Demarch Z, Giampa C, Patassini S et al (2007) Beneficial effects of rolipram in a quinolinic acid model of striatal excitotoxicity. Neurobiol Dis 25:266–273
DeMarch Z, Giampa C, Patassini S et al (2008) Beneficial effects of rolipram in the R6/2 mouse model of Huntington's disease. Neurobiol Dis 30:375–387
Denovan-Wright EM, Robertson HA (2000) Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. Neuroscience 98:705–713
Deuschl G, Schade-Brittinger C, Krack P et al (2006) A randomized trial of deep-brain stimulation for Parkinson's disease. N Engl J Med 355:896–908
Domenici MR, Scattoni ML, Martire A et al (2007) Behavioral and electrophysiological effects of the adenosine A2A receptor antagonist SCH 58261 in R6/2 Huntington's disease mice. Neurobiol Dis 28:197–205
Doria JG, Silva FR, de Souza JM et al (2013) Metabotropic glutamate receptor 5 positive allosteric modulators are neuroprotective in a mouse model of Huntington's disease. Br J Pharmacol 169:909–921

Dowie MJ, Bradshaw HB, Howard ML et al (2009) Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic mouse model of Huntington's disease. Neuroscience 163:456–465

Dowie MJ, Howard ML, Nicholson LF et al (2010) Behavioural and molecular consequences of chronic cannabinoid treatment in Huntington's disease transgenic mice. Neuroscience 170:324–336

Dubinsky R, Gray C (2006) CYTE-I-HD: phase I dose finding and tolerability study of cysteamine (Cystagon) in Huntington's disease. Mov Disord 21:530–533

Duff K, Paulsen JS, Beglinger LJ et al (2007) Psychiatric symptoms in Huntington's disease before diagnosis: the predict-HD study. Biol Psychiatry 62:1341–1346

Duff K, Paulsen JS, Beglinger LJ et al (2010) Frontal behaviors before the diagnosis of Huntington's disease and their relationship to markers of disease progression: evidence of early lack of awareness. J Neuropsychiatry Clin Neurosci 22:196–207

Dyhring T, Nielsen EO, Sonesson C et al (2010) The dopaminergic stabilizers pridopidine (ACR16) and (-)-OSU6162 display dopamine D(2) receptor antagonism and fast receptor dissociation properties. Eur J Pharmacol 628:19–26

Ehrnhoefer DE, Sutton L, Hayden MR (2011) Small changes, big impact: posttranslational modifications and function of huntingtin in Huntington disease. Neuroscientist 17:475–492

Eidelberg D, Surmeier DJ (2011) Brain networks in Huntington disease. J Clin Invest 121:484–492

Faideau M, Kim J, Cormier K et al (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet 19:3053–3067

Fasano A, Mazzone P, Piano C et al (2008) GPi-DBS in Huntington's disease: results on motor function and cognition in a 72-year-old case. Mov Disord 23:1289–1292

Fawcett AP, Moro E, Lang AE et al (2005) Pallidal deep brain stimulation influences both reflexive and voluntary saccades in Huntington's disease. Mov Disord 20:371–377

Ferrante A, Martire A, Armida M et al (2010) Influence of CGS 21680, a selective adenosine A(2A) receptor agonist, on NMDA receptor function and expression in the brain of Huntington's disease mice. Brain Res 1323:184–191

Figiel M, Szlachcic WJ, Switonski PM et al (2012) Mouse models of polyglutamine diseases: review and data table. Part I. Mol Neurobiol 46:393–429

Fischbeck KH (2001) Polyglutamine expansion neurodegenerative disease. Brain Res Bull 56:161–163

Fletcher JM, Hughes RA (2009) Modified low molecular weight cyclic peptides as mimetics of BDNF with improved potency, proteolytic stability and transmembrane passage in vitro. Bioorg Med Chem 17:2695–2702

Folstein SE, Chase GA, Wahl WE et al (1987) Huntington disease in Maryland: clinical aspects of racial variation. Am J Hum Genet 41:168–179

Forrest CM, Mackay GM, Stoy N et al (2010) Blood levels of kynurenines, interleukin-23 and soluble human leucocyte antigen-G at different stages of Huntington's disease. J Neurochem 112:112–122

Frank S (2010) Tetrabenazine: the first approved drug for the treatment of chorea in US patients with Huntington disease. Neuropsychiatr Dis Treat 6:657–665

Frank S, Ondo W, Fahn S et al (2008) A study of chorea after tetrabenazine withdrawal in patients with Huntington disease. Clin Neuropharmacol 31:127–133

Gellerich FN, Gizatullina Z, Nguyen HP et al (2008) Impaired regulation of brain mitochondria by extramitochondrial Ca2 + in transgenic Huntington disease rats. J Biol Chem 283:30715–30724

Gertz M, Fischer F, Nguyen GT et al (2013) Ex-527 inhibits Sirtuins by exploiting their unique NAD + -dependent deacetylation mechanism. Proc Natl Acad Sci U S A 110(30):E2772–781

Giampa C, Laurenti D, Anzilotti S et al (2010) Inhibition of the striatal specific phosphodiesterase PDE10A ameliorates striatal and cortical pathology in R6/2 mouse model of Huntington's disease. PLoS One 5:e13417

Giampa C, Montagna E, Dato C et al (2013) Systemic delivery of recombinant brain derived neurotrophic factor (BDNF) in the R6/2 mouse model of Huntington's disease. PLoS One 8:e64037

Giampà C, DeMarch Z, D'Angelo V et al (2006) Striatal modulation of cAMP-response-element-binding protein (CREB) after excitotoxic lesions: implications with neuronal vulnerability in Huntington's disease. Eur J Neurosci 23:11–20

Giampà C, Patassini S, Borreca A et al (2009a) Phosphodiesterase 10 inhibition reduces striatal excitotoxicity in the quinolinic acid model of Huntington's disease. Neurobiol Dis 34:450–456

Giampà C, Middei S, Patassini S et al (2009b) Phosphodiesterase type IV inhibition prevents sequestration of CREB binding protein, protects striatal parvalbumin interneurons and rescues motor deficits in the R6/2 mouse model of Huntington's disease. Eur J Neurosci 29:902–910

Gines S (2003) Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. Hum Mol Genet 12:497–508

Giralt A, Saavedra A, Carreton O et al (2011) Increased PKA signaling disrupts recognition memory and spatial memory: role in Huntington's disease. Hum Mol Genet 20(21):4232–4247

Giralt A, Carreton O, Lao-Peregrin C et al (2011b) Conditional BDNF release under pathological conditions improves Huntington's disease pathology by delaying neuronal dysfunction. Mol Neurodegener 6:71

Glass M, Faull RL, Dragunow M (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. Neuroscience 56:523–527

Glass M, Dragunow M, Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97:505–519

Goggi J, Pullar IA, Carney SL et al (2002) Modulation of neurotransmitter release induced by brain-derived neurotrophic factor in rat brain striatal slices in vitro. Brain Res 941:34–42

Gray MA, Egan GF, Ando A et al (2013) Prefrontal activity in Huntington's disease reflects cognitive and neuropsychiatric disturbances: the IMAGE-HD study. Exp Neurol 239:218–228

Grondin R, Kaytor MD, Ai Y et al (2012) Six-month partial suppression of Huntingtin is well tolerated in the adult rhesus striatum. Brain 135:1197–1209

Gu X, Li C, Wei W et al (2005) Pathological cell–cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron 46:433–444

Guenther MG, Barak O, Lazar MA (2001) The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Mol Cell Biol 21:6091–6101

Guidetti P, Reddy PH, Tagle DA et al (2000) Early kynurenergic impairment in Huntington's disease and in a transgenic animal model. Neurosci Lett 283:233–235

Guidetti P, Luthi-Carter RE, Augood SJ et al (2004) Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. Neurobiol Dis 17:455–461

Guidetti P, Bates GP, Graham RK et al (2006) Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice. Neurobiol Dis 23:190–197

Hall JA, Dominy JE, Lee Y et al (2013) The sirtuin family's role in aging and age-associated pathologies. J Clin Invest 123:973–979

Handa N, Mizohata E, Kishishita S et al (2008) Crystal Structure of the GAF-B Domain from Human Phosphodiesterase 10A Complexed with Its Ligand, cAMP. J Biol Chem 283:19657–19664

Haroon E, Raison CL, Miller AH (2012) Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37:137–162

Hassel B, Tessler S, Faull RL et al (2008) Glutamate uptake is reduced in prefrontal cortex in Huntington's disease. Neurochem Res 33:232–237

Hauser RA, Cantillon M, Pourcher E et al (2011) Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. Lancet Neurol 10:221–229

Hayden MR, MacGregor JM, Beighton PH (1980) The prevalence of Huntington's chorea in South Africa. S Afr Med J 58:193–196
Hebb A, Robertson H (2007) Role of phosphodiesterases in neurological and psychiatric disease. Curr Opin Pharmacol 7:86–92
Hebb MO, Garcia R, Gaudet P et al (2006) Bilateral stimulation of the globus pallidus internus to treat choreathetosis in Huntington's disease: technical case report. Neurosurgery 58:E383.discussion E383
Hebenstreit GF, Fellerer K, Fichte K et al (1989) Rolipram in major depressive disorder: results of a double-blind comparative study with imipramine. Pharmacopsychiatry 22:156–160
Heikkinen T, Lehtimaki K, Vartiainen N et al (2012) Characterization of neurophysiological and behavioral changes, MRI brain volumetry and 1H MRS in zQ175 knock-in mouse model of Huntington's disease. PLoS One 7:e50717
Hersch SM, Gevorkian S, Marder K et al (2006) Creatine in Huntington disease is safe, tolerable, bioavailable in brain and reduces serum 8OH2′dG. Neurology 66:250–252
Hickey MA, Zhu C, Medvedeva V et al (2012) Evidence for behavioral benefits of early dietary supplementation with CoEnzymeQ10 in a slowly progressing mouse model of Huntington's disease. Mol Cell Neurosci 49:149–157
Hilditch-Maguire P, Trettel F, Passani LA et al (2000) Huntingtin: an iron-regulated protein essential for normal nuclear and perinuclear organelles. Hum Mol Genet 9:2789–2797
Ho DJ, Calingasan NY, Wille E et al (2010) Resveratrol protects against peripheral deficits in a mouse model of Huntington's disease. Exp Neurol 225:74–84
Hockly E, Richon VM, Woodman B et al (2003) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci U S A 100:2041–2046
Hogel M, Laprairie RB, Denovan-Wright EM (2012) Promoters are differentially sensitive to N-terminal mutant huntingtin-mediated transcriptional repression. PLoS One 7:e41152
Horne EA, Coy J, Swinney K et al (2013) Downregulation of cannabinoid receptor 1 from neuropeptide Y interneurons in the basal ganglia of patients with Huntington's disease and mouse models. Eur J Neurosci 37:429–440
Houslay MD, Schafer P, Zhang KYJ (2005) Keynote review: phosphodiesterase-4 as a therapeutic target. Drug Discovery Today 10:1503–1519
Huang Z, Dias R, Jones T et al (2007) L-454,560, a potent and selective PDE4 inhibitor with in vivo efficacy in animal models of asthma and cognition. Biochem Pharmacol 73:1971–1981
Hubbard BP, Loh C, Gomes AP et al (2013) Carboxamide SIRT1 inhibitors block DBC1 binding via an acetylation-independent mechanism. Cell Cycle 12(14):2233–2240. doi:10.4161/cc.25268
Hult S, Schultz K, Soylu R et al (2010) Hypothalamic and neuroendocrine changes in Huntington's disease. Curr Drug Targets 11:1237–1249
Hunter A, Bordelon Y, Cook I et al. (2010) QEEG measures in Huntington's disease: a pilot study. PLoS Curr 2:RRN1192
Huntington Study Group (2001) A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. Neurology 57:397–404
Huntington Study Group (**2006**) Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. Neurology 66:366–372
Huntington Study Group TREND-HD Investigators (2008) Randomized controlled trial of ethyl-eicosapentaenoic acid in Huntington disease: the TREND-HD study. Arch Neurol 65:1582–1589
Huys D, Bartsch C, Poppe P et al (2013) Management and outcome of pallidal deep brain stimulation in severe Huntington's disease. Fortschr Neurol Psychiatr 81:202–205
Hyson HC, Kieburtz K, Shoulson I et al (2010) Safety and tolerability of high-dosage coenzyme Q10 in Huntington's disease and healthy subjects. Mov Disord 25:1924–1928
Imanishi T, Sawa A, Ichimaru Y et al (1997) Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. Eur J Pharmacol 321:273–278

Iona S, Cuomo M, Bushnik T et al (1998) Characterization of the rolipram-sensitive, cyclic AMP-specific phosphodiesterases: identification and differential expression of immunologically distinct forms in the rat brain. Mol Pharmacol 53:23–32

Ivkovic S, Ehrlich ME (1999) Expression of the striatal DARPP-32/ARPP-21 phenotype in GABAergic neurons requires neurotrophins in vivo and in vitro. J Neurosci 19:5409–5419

Jenkins BG, Rosas HD, Chen YC et al (1998) 1H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. Neurology 50:1357–1365

Jeon YH, Heo YS, Kim CM et al (2005) Phosphodiesterase: overview of protein structures, potential therapeutic applications and recent progress in drug development. Cell Mol Life Sci 62:1198–1220

Jeong H, Then F, Melia TJ Jr et al (2009) Acetylation targets mutant huntingtin to autophagosomes for degradation. Cell 137:60–72

Jeong H, Cohen DE, Cui L et al (2012) Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. Nat Med 18:159–165

Jia H, Kast RJ, Steffan JS et al (2012a) Selective histone deacetylase (HDAC) inhibition imparts beneficial effects in Huntington's disease mice: implications for the ubiquitin-proteasomal and autophagy systems. Hum Mol Genet 21:5280–5293

Jia H, Pallos J, Jacques V et al (2012b) Histone deacetylase (HDAC) inhibitors targeting HDAC3 and HDAC1 ameliorate polyglutamine-elicited phenotypes in model systems of Huntington's disease. Neurobiol Dis 46:351–361

Jiang M, Wang J, Fu J et al (2012) Neuroprotective role of Sirt1 in mammalian models of Huntington's disease through activation of multiple Sirt1 targets. Nat Med 18:153–158

Jiang M, Peng Q, Liu X et al (2013) Small-molecule TrkB receptor agonists improve motor function and extend survival in a mouse model of Huntington's disease. Hum Mol Genet 22:2462–2470

Jiao Y, Zhang Z, Zhang C et al (2011) A key mechanism underlying sensory experience-dependent maturation of neocortical GABAergic circuits in vivo. Proc Natl Acad Sci U S A 108:12131–12136

Jin J, Albertz J, Guo Z et al (2013) Neuroprotective effects of PPAR-gamma agonist rosiglitazone in N171-82Q mouse model of Huntington's disease. J Neurochem 125:410–419

Johnson JA, Johnson DA, Kraft AD et al (2008) The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. Ann N Y Acad Sci 1147:61–69

Johri A, Calingasan NY, Hennessey TM et al (2012) Pharmacologic activation of mitochondrial biogenesis exerts widespread beneficial effects in a transgenic mouse model of Huntington's disease. Hum Mol Genet 21:1124–1137

Jovicic A, Zaldivar Jolissaint JF, Moser R et al (2013) MicroRNA-22 (miR-22) overexpression is neuroprotective via general anti-apoptotic effects and may also target specific Huntington's disease-related mechanisms. PLoS One 8:e54222

Kang GA, Heath S, Rothlind J et al (2011) Long-term follow-up of pallidal deep brain stimulation in two cases of Huntington's disease. J Neurol Neurosurg Psychiatry 82:272–277

Kara E, Lin H, Svensson K et al (2010) Analysis of the actions of the novel dopamine receptor-directed compounds (S)-OSU6162 and ACR16 at the D2 dopamine receptor. Br J Pharmacol 161:1343–1350

Kegel KB, Sapp E, Alexander J et al (2009) Polyglutamine expansion in huntingtin alters its interaction with phospholipids. J Neurochem 110:1585–1597

Kells AP, Fong DM, Dragunow M et al (2004) AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Mol Ther 9:682–688

Khoshnan A, Patterson PH (2011) The role of IkappaB kinase complex in the neurobiology of Huntington's disease. Neurobiol Dis 43:305–311

Kim J, Amante DJ, Moody JP et al (2010) Reduced creatine kinase as a central and peripheral biomarker in Huntington's disease. Biochim Biophys Acta 1802:673–681

Kleiman RJ, Kimmel LH, Bove SE et al (2010) Chronic suppression of phosphodiesterase 10A alters striatal expression of Genes responsible for neurotransmitter synthesis,

neurotransmission, and signaling pathways implicated in Huntington's disease. J Pharmacol Exp Ther 336:64–76
Kleiman RJ, Kimmel LH, Bove SE et al (2011) Chronic suppression of phosphodiesterase 10A alters striatal expression of genes responsible for neurotransmitter synthesis, neurotransmission, and signaling pathways implicated in Huntington's disease. J Pharmacol Exp Ther 336:64–76
Kobayashi M, Kubo S, Iwata M et al (2011) ASP3258, an orally active potent phosphodiesterase 4 inhibitor with low emetic activity. Int Immunopharmacol 11:732–739
Konradsson-Geuken A, Wu HQ, Gash CR et al (2010) Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. Neuroscience 169:1848–1859
Kordasiewicz HB, Stanek LM, Wancewicz EV et al (2012) Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 74:1031–1044
Kotera J (2003) Subcellular localization of cyclic nucleotide phosphodiesterase type 10A variants, and alteration of the localization by cAMP-dependent protein kinase-dependent phosphorylation. J Biol Chem 279:4366–4375
Kuroiwa M, Snyder GL, Shuto T et al (2011) Phosphodiesterase 4 inhibition enhances the dopamine D1 receptor/PKA/DARPP-32 signaling cascade in frontal cortex. Psychopharmacology (Berl) 25(8):1101–1117
La Spada AR (2012) Finding a sirtuin truth in Huntington's disease. Nat Med 18:24–26
Landwehrmeyer GB, Dubois B, de Yebenes JG et al (2007) Riluzole in Huntington's disease: a 3-year, randomized controlled study. Ann Neurol 62:262–272
Langbehn DR, Hayden MR, Paulsen JS (2010) CAG-repeat length and the age of onset in Huntington disease (HD): a review and validation study of statistical approaches. Am J Med Genet B Neuropsychiatr Genet 153B:397–408
Lastres-Becker I, Berrendero F, Lucas JJ et al (2002) Loss of mRNA levels, binding and activation of GTP-binding proteins for cannabinoid CB1 receptors in the basal ganglia of a transgenic model of Huntington's disease. Brain Res 929:236–242
Lemtiri-Chlieh F, Levine ES (2010) BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. J Neurophysiol 104:1923–1932
Li YF, Cheng YF, Huang Y et al (2011) Phosphodiesterase-4D knock-out and RNA interference-mediated knock-down enhance memory and increase hippocampal neurogenesis via increased cAMP signaling. J Neurosci 31:172–183
Ligot N, Krystkowiak P, Simonin C et al (2011) External globus pallidus stimulation modulates brain connectivity in Huntington's disease. J Cereb Blood Flow Metab 31:41–46
Lin YS, Chen CM, Soong BW et al (2011) Dysregulated brain creatine kinase is associated with hearing impairment in mouse models of Huntington disease. J Clin Invest 121:1519–1523
Liot G, Zala D, Pla P et al (2013) Mutant Huntingtin alters retrograde transport of TrkB receptors in striatal dendrites. J Neurosci 33:6298–6309
Lopes C, Pereira EF, Wu HQ et al (2007) Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at alpha7* nicotinic receptors. J Pharmacol Exp Ther 322:48–58
Lundin A, Dietrichs E, Haghighi S et al (2010) Efficacy and safety of the dopaminergic stabilizer Pridopidine (ACR16) in patients with Huntington's disease. Clin Neuropharmacol 33:260–264
Luthi-Carter R, Taylor DM, Pallos J et al (2010) SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. Proc Natl Acad Sci U S A 107:7927–7932
Margolis RL, Ross CA (2001) Expansion explosion: new clues to the pathogenesis of repeat expansion neurodegenerative diseases. Trends Mol Med 7:479–482
Marongiu D, Imbrosci B, Mittmann T (2013) Modulatory effects of the novel TrkB receptor agonist 7,8-dihydroxyflavone on synaptic transmission and intrinsic neuronal excitability in mouse visual cortex in vitro. Eur J Pharmacol 709:64–71

Martin-Aparicio E, Yamamoto A, Hernandez F et al (2001) Proteasomal-dependent aggregate reversal and absence of cell death in a conditional mouse model of Huntington's disease. J Neurosci 21:8772–8781

Martire A, Ferrante A, Potenza RL et al (2010) Remodeling of striatal NMDA receptors by chronic A(2A) receptor blockade in Huntington's disease mice. Neurobiol Dis 37:99–105

Martire A, Pepponi R, Domenici MR et al (2013) BDNF prevents NMDA-induced toxicity in models of Huntington's disease: the effects are genotype specific and adenosine A(2A) receptor is involved. J Neurochem doi:10.1111/jnc.12177

Massa SM, Yang T, Xie Y et al (2010) Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest 120:1774–1785

Mazarei G, Budac DP, Lu G et al (2013) Age-dependent alterations of the kynurenine pathway in the YAC128 mouse model of Huntington disease. J Neurochem doi: 10.1111/jnc.12350

McFarland KN, Das S, Sun TT et al (2012) Genome-wide histone acetylation is altered in a transgenic mouse model of Huntington's disease. PLoS One 7:e41423

Mehvar R, Jamali F, Watson MW et al (1987) Pharmacokinetics of tetrabenazine and its major metabolite in man and rat: Bioavailability and dose dependency studies. Drug Metab Dispos 15:250–255

Menalled LB, Patry M, Ragland N et al (2010) Comprehensive behavioral testing in the R6/2 mouse model of Huntington's disease shows no benefit from CoQ10 or minocycline. PLoS One 5:e9793

Mielcarek M, Benn CL, Franklin SA et al (2011) SAHA decreases HDAC 2 and 4 levels in vivo and improves molecular phenotypes in the R6/2 mouse model of Huntington's disease. PLoS One 6:e27746

Mievis S, Blum D, Ledent C (2011) Worsening of Huntington disease phenotype in CB1 receptor knockout mice. Neurobiol Dis 42:524–529

Miller JP, Hughes RE (2011) Protein interactions and target discovery in Huntington's disease. In: Lo DC, Hughes RE (eds) Neurobiology of Huntington's disease. Applications to drug discovery. CRC, Boca Raton

Miller BR, Dorner JL, Bunner KD et al (2012) Up-regulation of GLT1 reverses the deficit in cortically evoked striatal ascorbate efflux in the R6/2 mouse model of Huntington's disease. J Neurochem 121:629–638

Milnerwood AJ, Kaufman AM, Sepers MD et al (2012) Mitigation of augmented extrasynaptic NMDAR signaling and apoptosis in cortico-striatal co-cultures from Huntington's disease mice. Neurobiol Dis 48:40–51

Mishra R, Hoop CL, Kodali R et al (2012) Serine phosphorylation suppresses huntingtin amyloid accumulation by altering protein aggregation properties. J Mol Biol 424:1–14

Mitchell CJ, Ballantine SP, Coe DM et al (2010) Pyrazolopyridines as potent PDE4B inhibitors: 5-heterocycle SAR. Bioorg Med Chem Lett 20:5803–5806

Miyake N, Skinbjerg M, Easwaramoorthy B et al (2011) Imaging changes in glutamate transmission in vivo with the metabotropic glutamate receptor 5 tracer [11C] ABP688 and N-acetylcysteine challenge. Biol Psychiatry 69:822–824

Mizuno Y, Hasegawa K, Kondo T et al (2010) Clinical efficacy of istradefylline (KW-6002) in Parkinson's disease: a randomized, controlled study. Mov Disord 25:1437–1443

Mochel F, Haller RG (2011) Energy deficit in Huntington disease: why it matters. J Clin Invest 121:493–499

Mochel F, Duteil S, Marelli C et al (2010) Dietary anaplerotic therapy improves peripheral tissue energy metabolism in patients with Huntington's disease. Eur J Hum Genet 18:1057–1060

Mochel F, Durant B, Meng X et al (2012) Early alterations of brain cellular energy homeostasis in Huntington disease models. J Biol Chem 287:1361–1370

Mok MH, Fricker AC, Weil A et al (2009) Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels. Neuropharmacology 57:242–249

Moro E, Lang AE, Strafella AP et al (2004) Bilateral globus pallidus stimulation for Huntington's disease. Ann Neurol 56:290–294

Moumne L, Campbell K, Howland D et al (2012) Genetic knock-down of HDAC3 does not modify disease-related phenotypes in a mouse model of Huntington's disease. PLoS One 7:e31080

Munoz-Sanjuan I, Bates GP (2011) The importance of integrating basic and clinical research toward the development of new therapies for Huntington disease. J Clin Invest 121:476–483

Nagakura A, Niimura M, Takeo S (2002) Effects of a phosphodiesterase IV inhibitor rolipram on microsphere embolism-induced defects in memory function and cerebral cyclic AMP signal transduction system in rats. Br J Pharmacol 135:1783–1793

Naganuma K, Omura A, Maekawara N et al (2009) Discovery of selective PDE4B inhibitors. Bioorg Med Chem Lett 19:3174–3176

Nin V, Escande C, Chini CC et al (2012) Role of deleted in breast cancer 1 (DBC1) protein in SIRT1 deacetylase activation induced by protein kinase A and AMP-activated protein kinase. J Biol Chem 287:23489–23501

Nishi A, Watanabe Y, Higashi H et al (2005) Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. Proc Natl Acad Sci U S A 102:1199–1204

Obrietan K (2004) CRE-mediated transcription is increased in Huntington's disease transgenic mice. J Neurosci 24:791–796

Okuda S, Nishiyama N, Saito H et al (1998) 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J Neurochem 70:299–307

O'Leary PD, Hughes RA (2003) Design of potent peptide mimetics of brain-derived neurotrophic factor. J Biol Chem 278:25738–25744

Orru M, Zanoveli JM, Quiroz C et al (2011a) Functional changes in postsynaptic adenosine A(2A) receptors during early stages of a rat model of Huntington disease. Exp Neurol 232:76–80

Orru M, Bakesova J, Brugarolas M et al (2011b) Striatal pre- and postsynaptic profile of adenosine A(2A) receptor antagonists. PLoS One 6:e16088

Paleacu D (2007) Tetrabenazine in the treatment of Huntington's disease. Neuropsychiatr Dis Treat 3:545–551

Perreault M, Feng G, Will S et al (2013) Activation of TrkB with TAM-163 results in opposite effects on body weight in rodents and non-human primates. PLoS One 8:e62616

Pettibone DJ, Totaro JA, Pflueger AB (1984a) Tetrabenazine-induced depletion of brain monoamines: characterization and interaction with selected antidepressants. Eur J Pharmacol 102:425–430

Pettibone DJ, Pflueger AB, Totaro JA (1984b) Tetrabenazine-induced depletion of brain monoamines: mechanism by which desmethylimipramine protects cortical norepinephrine. Eur J Pharmacol 102:431–436

Pisani A, Bernardi G, Ding J et al (2007) Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30:545–553

Pocivavsek A, Wu HQ, Potter MC et al (2011) Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. Neuropsychopharmacology 36:2357–2367

Ponten H, Kullingsjo J, Lagerkvist S et al (2010) In vivo pharmacology of the dopaminergic stabilizer pridopidine. Eur J Pharmacol 644:88–95

Popoli P, Blum D, Domenici MR et al (2008) A critical evaluation of adenosine A2A receptors as potentially druggable targets in Huntington's disease. Curr Pharm Des 14:1500–1511

Puri BK, Bydder GM, Counsell SJ et al (2002) MRI and neuropsychological improvement in Huntington disease following ethyl-EPA treatment. Neuroreport 13:123–126

Puri BK, Leavitt BR, Hayden MR et al (2005) Ethyl-EPA in Huntington disease: a double-blind, randomized, placebo-controlled trial. Neurology 65:286–292

Qin ZH, Wang Y, Sapp E et al (2004) Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. J Neurosci 24:269–281

Quinti L, Chopra V, Rotili D et al (2010) Evaluation of histone deacetylases as drug targets in Huntington's disease models: study of HDACs in brain tissues from R6/2 and CAG140

knock-in HD mouse models and human patients and in a neuronal HD cell model. PLoS Curr 2: pii:RRN1172

Raynes R, Pombier KM, Nguyen K et al (2013) The SIRT1 modulators AROS and DBC1 regulate HSF1 activity and the heat shock response. PLoS One 8:e54364

Reiner A, Dragatsis I, Zeitlin S et al (2003) Wild-type huntingtin plays a role in brain development and neuronal survival. Mol Neurobiol 28:259–276

Reiner A, Lafferty DC, Wang HB et al (2012) The group 2 metabotropic glutamate receptor agonist LY379268 rescues neuronal, neurochemical and motor abnormalities in R6/2 Huntington's disease mice. Neurobiol Dis 47:75–91

Reynolds NC, Prost RW, Mark LP et al (2008) MR-spectroscopic findings in juvenile-onset Huntington's disease. Mov Disord 23:1931–1935

Ribeiro FM, Paquet M, Ferreira LT et al (2010) Metabotropic glutamate receptor-mediated cell signaling pathways are altered in a mouse model of Huntington's disease. J Neurosci 30:316–324

Richfield EK, Herkenham M (1994) Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. Ann Neurol 36:577–584

Roberts MS, McLean S, Millingen KS et al (1986) The pharmacokinetics of tetrabenazine and its hydroxy metabolite in patients treated for involuntary movement disorders. Eur J Clin Pharmacol 29:703–708

Robichaud A, Tattersall FD, Choudhury I et al (1999) Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret. Neuropharmacology 38:289–297

Robichaud A, Savoie C, Stamatiou PB et al (2002) Assessing the emetic potential of PDE4 inhibitors in rats. Br J Pharmacol 135:113–118

Rodefer JS, Saland SK, Eckrich SJ (2012) Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. Neuropharmacology 62(3):1182-1190

Rose GM, Hopper A, De Vivo M et al (2005) Phosphodiesterase inhibitors for cognitive enhancement. Curr Pharm Des 11:3329–3334

Ross CA, Tabrizi SJ (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 10:83–98

Rung JP, Rung E, Helgeson L et al (2008) Effects of (-)-OSU6162 and ACR16 on motor activity in rats, indicating a unique mechanism of dopaminergic stabilization. J Neural Transm 115:899–908

Rutten K, Prickaerts J, Blokland A (2006) Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. Neurobiol Learn Mem 85:132–138

Rutten K, Lieben C, Smits L et al (2007a) The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. Psychopharmacology (Berl) 192:275–282

Rutten K, Prickaerts J, Hendrix M et al (2007b) Time-dependent involvement of cAMP and cGMP in consolidation of object memory: studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. Eur J Pharmacol 558:107–112

Rutten K, Basile JL, Prickaerts J et al (2008) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. Psychopharmacology (Berl) 196:643–648

Sadri-Vakili G, Bouzou B, Benn CL et al (2007) Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. Hum Mol Genet 16:1293–1306

Sah DW, Aronin N (2011) Oligonucleotide therapeutic approaches for Huntington disease. J Clin Invest 121:500–507

Sanberg PR, Fibiger HC, Mark RF (1981) Body weight and dietary factors in Huntington's disease patients compared with matched controls. Med J Aust 1:407–409

Sapko MT, Guidetti P, Yu P et al (2006) Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. Exp Neurol 197:31–40

Sari Y, Prieto AL, Barton SJ et al (2010) Ceftriaxone-induced up-regulation of cortical and striatal GLT1 in the R6/2 model of Huntington's disease. J Biomed Sci 17:62

Sathasivam K, Lane A, Legleiter J et al (2010) Identical oligomeric and fibrillar structures captured from the brains of R6/2 and knock-in mouse models of Huntington's disease. Hum Mol Genet 19:65–78

Sathasivam K, Neueder A, Gipson TA et al (2013) Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc Natl Acad Sci U S A 110:2366–2370

Sathyasaikumar KV, Stachowski EK, Amori L et al (2010) Dysfunctional kynurenine pathway metabolism in the R6/2 mouse model of Huntington's disease. J Neurochem 113:1416–1425

Schiefer J, Sprunken A, Puls C et al (2004) The metabotropic glutamate receptor 5 antagonist MPEP and the mGluR2 agonist LY379268 modify disease progression in a transgenic mouse model of Huntington's disease. Brain Res 1019:246–254

Schmidt CJ, Chapin DS, Cianfrogna J et al (2008) Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Ther 325:681–690

Schwarcz R, Bruno JP, Muchowski PJ et al (2012) Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci 13:465–477

Sebastian C, Satterstrom FK, Haigis MC et al (2012) From sirtuin biology to human diseases: an update. J Biol Chem 287:42444–42452

Seeger T (2003) Immunohistochemical localization of PDE10A in the rat brain. Brain Res 985:113–126

Seong IS, Ivanova E, Lee JM et al (2005) HD CAG repeat implicates a dominant property of huntingtin in mitochondrial energy metabolism. Hum Mol Genet 14:2871–2880

Shin BH, Lim Y, Oh HJ et al (2013) Pharmacological activation of Sirt1 Ameliorates Polyglutamine-induced toxicity through the regulation of autophagy. PLoS One 8:e64953

Shirasaki DI, Greiner ER, Al-Ramahi I et al (2012) Network organization of the huntingtin proteomic interactome in mammalian brain. Neuron 75:41–57

Skaper SD, Di Marzo V (2012) Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philos Trans R Soc Lond B Biol Sci 367:3193–3200

Soldati C, Bithell A, Conforti P et al (2011) Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease. J Neurochem 116:415–425

Sotty F, Montezinho LP, Steiniger-Brach B et al (2009) Phosphodiesterase 10A inhibition modulates the sensitivity of the mesolimbic dopaminergic system to d-amphetamine: involvement of the D1-regulated feedback control of midbrain dopamine neurons. J Neurochem 109:766–775

Spina D (2008) PDE4 inhibitors: current status. Br J Pharmacol 155:308–315

Squitieri F, Landwehrmeyer B, Reilmann R et al (2013) One-year safety and tolerability profile of pridopidine in patients with Huntington disease. Neurology 80:1086–1094

Steffan JS, Bodai L, Pallos J et al (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature 413:739–743

Stiles DK, Zhang Z, Ge P et al (2012) Widespread suppression of huntingtin with convection-enhanced delivery of siRNA. Exp Neurol 233:463–471

Stone TW, Darlington LG (2002) Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov 1:609–620

Sugars KL (2003) Decreased cAMP Response Element-mediated Transcription: an early event in exon 1 and full-length cell models of Huntington's disease that contributes to polyglutamine pathogenesis. J Biol Chem 279:4988–4999

Switonski PM, Szlachcic WJ, Gabka A et al (2012) Mouse models of polyglutamine diseases in therapeutic approaches: review and data table. Part II. Mol Neurobiol 46:430–466

Tabrizi SJ, Blamire AM, Manners DN et al (2003) Creatine therapy for Huntington's disease: clinical and MRS findings in a 1-year pilot study. Neurology 61:141–142

Tam S, Spiess C, Auyeung W et al (2009) The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. Nat Struct Mol Biol 16:1279–1285

Tang JK, Moro E, Lozano AM et al (2005) Firing rates of pallidal neurons are similar in Huntington's and Parkinson's disease patients. Exp Brain Res 166:230–236

Tang B, Seredenina T, Coppola G et al (2011) Gene expression profiling of R6/2 transgenic mice with different CAG repeat lengths reveals genes associated with disease onset and progression in Huntington's disease. Neurobiol Dis 42:459–467

Tarditi A, Camurri A, Varani K et al (2006) Early and transient alteration of adenosine A2A receptor signaling in a mouse model of Huntington disease. Neurobiol Dis 23:44–53

Temel Y, Cao C, Vlamings R et al (2006) Motor and cognitive improvement by deep brain stimulation in a transgenic rat model of Huntington's disease. Neurosci Lett 406:138–141

Thakur AK, Jayaraman M, Mishra R et al (2009) Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. Nat Struct Mol Biol 16:380–389

The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Thomas EA, Coppola G, Desplats PA et al (2008) The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. Proc Natl Acad Sci U S A 105:15564–15569

Threlfell S, Sammut S, Menniti FS et al (2008) Inhibition of phosphodiesterase 10A increases the responsiveness of striatal projection neurons to cortical stimulation. J Pharmacol Exp Ther 328:785–795

Threlfell S, Sammut S, Menniti FS et al (2009) Inhibition of phosphodiesterase 10A increases the responsiveness of striatal projection neurons to cortical stimulation. J Pharmacol Exp Ther 328:785–795

Trevino RS, Lauckner JE, Sourigues Y et al (2012) Fibrillar structure and charge determine the interaction of polyglutamine protein aggregates with the cell surface. J Biol Chem 287:29722–29728

Tsao D, Thomsen HK, Chou J et al (2008) TrkB agonists ameliorate obesity and associated metabolic conditions in mice. Endocrinol 149:1038–1048

van den Bogaard SJ, Dumas EM, Teeuwisse WM et al (2011) Exploratory 7-Tesla magnetic resonance spectroscopy in Huntington's disease provides in vivo evidence for impaired energy metabolism. J Neurol 258:2230–2239

Van Laere K, Casteels C, Dhollander I et al (2010) Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. J Nucl Med 51:1413–1417

Van Raamsdonk JM, Pearson J, Rogers DA et al (2005) Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Exp Neurol 196:266–272

Vanevski F, Xu B (2013) Molecular and neural bases underlying roles of BDNF in the control of body weight. Front Neurosci 7:37

Vecsei L, Szalardy L, Fulop F et al (2013) Kynurenines in the CNS: recent advances and new questions. Nat Rev Drug Discov 12:64–82

Velez-Lago FM, Thompson A, Oyama G et al (2013) Differential and better response to deep brain stimulation of chorea compared to dystonia in Huntington's Disease. Stereotact Funct Neurosurg 91:129–133

Venuto CS, McGarry A, Ma Q et al (2012) Pharmacologic approaches to the treatment of Huntington's disease. Mov Disord 27:31–41

Verbessem P, Lemiere J, Eijnde BO et al (2003) Creatine supplementation in Huntington's disease: a placebo-controlled pilot trial. Neurology 61:925–930

Vlamings R, Benazzouz A, Chetrit J et al (2012) Metabolic and electrophysiological changes in the basal ganglia of transgenic Huntington's disease rats. Neurobiol Dis 48:488–494

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384

Wang ZM, Lashuel HA (2013) Discovery of a novel aggregation domain in the huntingtin protein: implications for the mechanisms of Htt aggregation and toxicity. Angew Chem Int Ed Engl 52:562–567

Wang H, Peng MS, Chen Y et al (2007) Structures of the four subfamilies of phosphodiesterase-4 provide insight into the selectivity of their inhibitors. Biochem J 408:193–201

Waterhouse EG, Xu B (2013) The skinny on brain-derived neurotrophic factor: evidence from animal models to GWAS. J Mol Med (Berl) 91(11):1241–1247. doi:10.1007/s00109-013-1071-8. Epub 2013 Jul 5

Weiss A, Trager U, Wild EJ et al (2012) Mutant huntingtin fragmentation in immune cells tracks Huntington's disease progression. J Clin Invest 122:3731–3736

Wen YD, Perissi V, Staszewski LM et al (2000) The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci U S A 97:7202–7207

Wexler NS, Lorimer J, Porter J et al (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 101:3498–3503

Weydt P, Pineda VV, Torrence AE et al (2006) Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab 4:349–362

Woda JM, Calzonetti T, Hilditch-Maguire P et al (2005) Inactivation of the Huntington's disease gene (Hdh) impairs anterior streak formation and early patterning of the mouse embryo. BMC Dev Biol 5:17

Wolf RC, Thomann PA, Thomann AK et al (2013) Brain structure in preclinical Huntington's disease: a multi-method approach. Neurodegener Dis 12(1):13–22

Wright HH, Still CN, Abramson RK (1981) Huntington's disease in black kindreds in South Carolina. Arch Neurol 38:412–414

Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. J Neurosci 30:14708–14718

Yamamoto A, Lucas JJ, Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101:57–66

Yang L, Calingasan NY, Wille EJ et al (2009) Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases. J Neurochem 109:1427–1439

Zala D, Colin E, Rangone H et al (2008) Phosphorylation of mutant huntingtin at S421 restores anterograde and retrograde transport in neurons. Hum Mol Genet 17:3837–3846

Zhang HT, Huang Y, Masood A et al (2008) Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). Neuropsychopharmacol 33:1611–1623

Zhang SF, Hennessey T, Yang L et al (2011) Impaired brain creatine kinase activity in Huntington's disease. Neurodegener Dis 8:194–201

Zhao W, Kruse JP, Tang Y et al (2008) Negative regulation of the deacetylase SIRT1 by DBC1. Nature 451:587–590

Zuccato C, Valenza M, Cattaneo E (2010) Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 90:905–981

Zuccato C, Marullo M, Vitali B et al (2011) Brain-derived neurotrophic factor in patients with Huntington's disease. PLoS One 6:e22966

Zwilling D, Huang SY, Sathyasaikumar KV et al (2011) Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. Cell 145:863–874

Part II
Behavioral Neurobiology of Parkinson's Disease

Clinical and Pathological Features of Parkinson's Disease

Susanne A. Schneider and Jose A. Obeso

Abstract Parkinson's disease (PD) is, after Alzheimer's disease, the second most common neurodegenerative disorder with an approximate prevalence of 0.5–1 % among persons 65–69 years of age, rising to 1–3 % among persons 80 years of age and older. Pathologically, PD is characterized by the loss of neurons in the substantia nigra pars compacta (SNpc), and by the presence of eosinophilic protein deposits (Lewy bodies) in this region, in other aminergic nuclei and in cortical and limbic structures. Moreover, it has now been shown that pathology also involves the peripheral nervous system. Braak and colleagues suggested a thread of pathology starting from the vagal nerve to progress to the brainstem, and eventually to limbic and neocortical brain regions. This progression of pathology may account for the clinical evolution of PD toward a composite symptomatology. However, this hypothesis has been criticized by others. In this chapter, we review the clinical features of PD (motor and nonmotor) and their pathological correlates.

Keywords Parkinson's disease · Pathology · Lewy bodies · Braak staging · Spread · Pathological correlate · Nonmotor · Motor

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S. A. Schneider (✉)
University of Kiel, Kiel, Germany
e-mail: s.schneider@neurologie.uni-kiel.de

J. A. Obeso
Department of Neurology and Neuroscience, Medical School,
Clinica Universitaria and CIMA, University of Navarra and CIBERNED,
Pamplona, Spain
e-mail: jobeso52@gmail.com

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1 Introduction

Parkinson's disease (PD) is a neurodegenerative disorder clinically characterized by levodopa-responsive slowness (bradykinesia), rigidity, tremor, and impaired postural stability (Donaldson et al. 2012). Brain pathology shows loss of neurons in the substantia nigra pars compacta (SNpc), with the presence of eosinophilic protein deposits (Lewy bodies) in the cytoplasm, and dopamine (DA) striatal depletion. These essential pathochemical characteristics account for the cardinal features of PD. It has been estimated that typical motor features appear when at least 50 % of nigral DA neurons and 70 % of putaminal DA tissue contents have been lost (Kordower et al. 2013; Fearnley and Lees 1991).

After Alzheimer's disease, PD is the second most common neurodegenerative disorder with an approximate prevalence of 0.5–1 % among persons 65–69 years of age, rising to 1–3 % among persons 80 years of age and older. In view of the aging population, the number of PD cases are expected to increase considerably over the next years. Thus, it is calculated that the number of individuals with PD over the age of 50 years will increase in Western Europe's five most and the world's ten most populous nations and rise to double from about 4 million in 2005 to some 8.7–9.3 million by 2030 (Dorsey et al. 2007).

PD presents sporadically, by and large, and cannot be considered a typically inherited condition. However, around 15 % of individuals with PD have a first-degree relative also affected by PD. In recent years, several monogenic causes and genetic risk factors of PD have been identified which account for about 5 % of PD patients (Gasser 2010). Interestingly, most genes so far associated with genetic PD are related to mitochondrial function but also the lysosomes and autophagy system (Surmeier and Schumacker 2013). For example, mutations in Parkin, encoding for a mitochondrial protein, is the most common cause of early onset PD (defined by an onset age of 40 years or earlier) (Bonifati 2012). Indeed, not all patients develop PD in late adulthood, but early onset cases have been well recognized and are more likely to have a genetic cause. On the other hand, the most extensively studied PD-related autosomal dominantly inherited genes include synuclein

(SNCA; which is important in PD because the alpha-synuclein protein is the main component of Lewy bodies) and glucocerebrosidase (GBA), the latter of which is also the most important risk factor for sporadic PD, increasing the risk for developing the disease up to 5–13-fold in certain ethnic groups (Sidransky and Lopez 2012; Beavan and Schapira 2013).

The pathological basis of PD has received large attention in recent years. Traditional studies had focused on the SNpc and the nigrostriatal dopaminergic projection. However, the discovery that Lewy bodies are constituted, among many other proteins, by ubiquitin and alpha-synuclein aggregates have substantially modified the views of PD pathology. Thus, excellent immunostaining techniques for these two proteins currently allow recognition of Lewy bodies not only in the SNpc but also in many other regions of the central and peripheral nervous system. This has led to suggest a thread of pathology in a caudo-rostral distribution (Braak et al. 2003; Wolters and Braak 2006). Alpha-synuclein pathology supposedly progresses from the caudal brainstem and olfactory bulb (in Braak stages 1–3) to subsequently spread to limbic and neocortical brain regions (in Braak stages 4–6) (Braak et al. 2003) (Fig. 1). Involvement of the vagal nerve has been hypothesized as the first pathological correlate by Braak and colleagues, and recent studies in mice suggest that resection of the autonomic nerves stops transneuronal and retrograde axonal transport of alpha-synuclein from the enteric nervous system into the brain (Pan-Montojo et al. 2012). These recent experimental studies are keeping in with Braak et al.'s original idea that synuclein pathology might first occur in the gastrointestinal tract and then slowly move into the central nervous system. Currently, a large degree of interest, if not enthusiasm, is being put into this concept. However, it is important to call attention to the fact that many aspects of Braak's hypothesis remain to be directly proven, and that Lewy bodies as such are not yet known to disrupt cell physiology. Indeed, whether or not Lewy bodies help neurons to resist the underlying neurodegenerative mechanisms and are therefore protective, or are causal to the disease process, ultimately killing neurons, is yet undeciphered. Accordingly, the above summarized findings should be seen as *state-of-the-art* thinking and researching in PD etiopathogenesis cannot be considered solid factual data.

2 Clinical Features of PD

Traditionally, PD is perceived as a movement disorder; the main features being slowness of movements, tremor, and muscle rigidity affecting one body part at onset.

With disease progression over the years, the motor features become more generalized, the trunk tends to bend forward, balance is impaired and walking becomes difficult (Litvan et al. 2003). These cardinal features of PD respond very well to levodopa and other dopaminergic drugs. The capacity to respond to dopaminergic drugs is another essential feature of PD albeit it is not specific, as

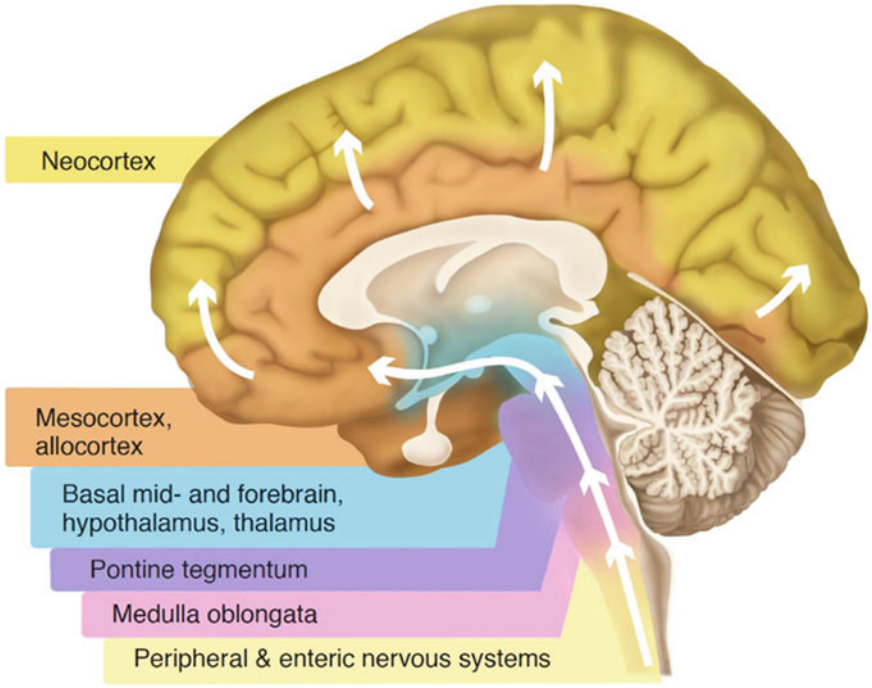

Fig. 1 Stages in the development of PD-related pathology—the Braak Hypothesis. Staging of Lewy pathology according to the Braak model. Schematic summarizing the progression of Parkinson's disease as proposed by Braak et al. (2003). According to the Braak model, αsyn deposits in specific brain regions and neuronal types giving rise to Lewy pathology in a stereotypic, temporal pattern that ascends caudo-rostrally from the lower brainstem (including the dorsal motor nucleus of the vagus nerve in the medulla then the coeruleus-subcoeruleus complex, raphe nuclei, gigantocellular reticular nucleus in the medulla a and pons) through susceptible regions of the midbrain (substantia nigra and the pedunculopontine tegmental nucleus) and forebrain (e.g., amygdala) and into the cerebral cortex (e.g., anteromedial temporal mesocortex, cingulate cortex, and later neocortical structures). It is hypothesized that the disease initiates in the periphery, gaining access to the CNS through retrograde transport along projection neurons from the gastrointestinal tract. As the disease progresses, the severity of lesions in the susceptible regions increases (Visanji et al. 2013)

any other pathological process producing lesion of the SNpc and DA depletion may equally respond in the same manner.

In recent years it has become more and more clear that PD is essentially a multisystem disorder presenting with numerous nonmotor features which may be present early in the evolution but typically become more prominent and disabling after many years of disease progression (Löhle et al. 2009).

The diagnosis of PD is made clinically. Diagnostic tests in vivo may support the diagnosis (for example, reduced uptake on dopaminergic SPECT/PET imaging, see below) but are mainly helpful to exclude secondary and other causes.

Postmortem brain pathology can confirm the diagnosis retrospectively (Donaldson et al. 2012).

In the following, we will give an overview of the main motor manifestations and some other clinical (non-motor) manifestations in PD, and a brief review of their pathophysiological basis.

3 Motor Features in PD

According to the Queen Square Brain Bank Criteria, slowness of movement is a *sine qua non* criterion for the diagnosis of PD (Lees et al. 2009) (Tables 1, 2, 3). In this context, bradykinesia refers to slowness of repetitive movements; to be separated from absence or poverty of expected spontaneous voluntary movement including slow reaction time (akinesia) and small amplitude movements (hypokinesia). In the clinical situation, these terms are often exchangeable (Lees et al. 2009). On physical examination, such slowness of voluntary movements may be observed as reduced degree of facial expression (hypomimia) and lack of spontaneous limb or trunk movements. Repetitive movements (such as finger or foot tapping) may be slow and become progressively reduced in amplitude, as if associated with fatigue. When walking patients may demonstrate general slowness with a small stepped gait, slow turning, and reduced (asymmetric) arm swing. The latter may be associated with (unilateral) shoulder pain, which may be one of the earliest symptoms, found in 35 of 309 consecutive PD patients preceding the onset of ipsilateral signs of motor parkinsonism (Stamey et al. 2008; Truong and Wolters 2009).

In later stages walking may be further impaired, by short steps, shuffling and "freezing episodes" (a sudden inability to move the lower extremities which may be triggered by tight, cluttered spaces or when attempting to turn) associated with a risk of falling. On other occasions patients may develop festination, a gait that becomes progressively faster while the patient bends forward and ends by falling, unless someone intercept the action.

The term tremor refers to a rhythmic sinusoidal movement of a body segment. In PD, tremor typically presents at rest, i.e., upon relaxation, affecting the hands characteristically as pill rolling tremor between the thumb and index finger. Rest tremor of the hand may also be triggered when walking. On action (for example, when being asked to lift the arms to keep them outstretched) the tremor typically pauses for a few seconds, followed by re-emergence of the same tremor activity. This phenomenon is rather typical of PD (i.e., absent in other types of tremor such as essential tremor). Tremor may also affect forearms, legs, the head, or jaw. The typical frequency of PD tremor is 4–6 Hz.

Both tremor and bradykinesia contribute to impaired fine motor dexterity and motor coordination which is often one of the first complaints, for example, manifesting with small, untidy handwriting (micrographia).

Table 1 Queen Square Brain Bank criteria clinical diagnostic criteria for PD (from Litvan et al. 2003)

Inclustion criteria
Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)
And at least one of the following:
Muscular rigidity
4–6 Hz rest tremor
Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

PD Parkinson disease

Table 2 Supportive prospective positive criteria of Parkinson's disease (from Lees et al. 2009)

Unilateral onset	Clinical course of 10 years or more
Rest tremor present	Hyposmia
Progressive disorder	Visual hallucination
Persistent asymmetry affecting predominantly the side of onset	
Excellent response (70–100 %) to L-dopa	
Severe L-dopa-induced chorea	
L-dopa response for 5 years or more	

Three or more required for diagnosis of definite Parkinson's disease

Table 3 Exclusion criteria for Parkinson's disease (from Lees et al. 2009)

Exclusion criteria for Parkinson's disease
• History of repeated strokes with stepwise progression of parkinsonian features
• History of repeated head injury
• History of definite encephalitis
• Oculogyric crises
• Neuroleptic treatment at onset of symptoms
• More than one affected relative
• Sustained remission
• Strictly unilateral features after 3 years
• Supranuclear gaze palsy
• Cerebellar signs
• Early severe autonomic involvement
• Early severe dementia with disturbances of memory, language and praxis
• Babinski sign
• Presence of a cerebral tumor or communicating hydrocephalus on CT scan
• Negative response to large doses of L-dopa (if malabsorption excluded)
• MPTP exposure

CT Computed tomography

Rigidity means increased muscle tone perceived by the examiner as resistance to passive displacement of a given joint. Patients feel stiff and frequently complain of muscular pain.

Based on the combination of these signs, two broad types of PD have been recognized: an akinetic-rigid and a tremor-dominant form. Pathologically, as mentioned above, motor signs are mainly attributed to loss of dopaminergic neurons in the substantia nigra. Some studies tried to correlate the clinical subtype with specific changes on brain pathology, but data are conflicting. Similarly, attempts have been made to demonstrate the degree of involvement of the nigrostriatal system as the disease progresses, thus at varying time points after diagnosis. In this regard, most reviews estimate a loss of 50–70 % of SNc dopamine neurons at the time when symptoms emerge (Kordower et al. 2013). With regard to disease progression, it has been suggested that patients with the akinetic-rigid subtype have a greater risk to evolve toward dementia and other nonmotor problems, as they may exhibit more severe cortical Lewy body pathology and higher Braak stages than tremor-dominant patients (Selikhova et al. 2009).

4 Motor Complications of PD Pharmacotherapy

The classic motor features of PD respond very well to dopaminergic replacement therapy, i.e., dopamine agonists and levodopa. As disease progresses, higher medication doses are needed, presumably because the degree of striatal DA depletion increases. The combination of larger nigrostriatal deficit and high (>600 mg/day) levodopa doses leads to motor complications with a variety of dyskinetic movements in patients with PD, all of which are highly disabling (Thanvi et al. 2007; Ondo 2011). Choreic involuntary movements occurring as peak-time dyskinesia are the most common form. They are related to peak plasma levels of levodopa. Diphasic dyskinesias (commonly dystonic in nature) develop when plasma levodopa levels are rising or falling. Motor complications may also manifest as "*off*" state dystonia, which occur when plasma levodopa levels are low (i.e., in the early morning). These problems are currently treated with fair efficacy by using continuous delivery of levodopa (intra-duodenally) or apomorphine subcutaneously and by deep brain stimulation or restricted lesion of the globus pallidus ((GP), i.e. pallidotomy) (reviewed in Cenci et al. 2011).

The incidence of levodopa-induced dyskinesia (LID) in PD ranges between 9 and 80 % depending on several factors, such as age, disease severity, levodopa dosage, and treatment duration (reviewed in Manson et al. 2012). Early onset PD patients have a higher risk of developing motor complications. The pathogenesis of LID remains incompletely understood but no doubt it is the combination of a degree of nigrostriatal denervation and the effect of levodopa that plays a major role and determines the onset of LID (Voon et al. 2009; Cenci and Lundblad 2006). A key pathogenic factor in LID is the levodopa daily dose, with a threshold around 400–500 mg (Olanow et al. 2013).

Pathophysiologically, choreic dyskinesias are associated with decreased neuronal firing in the globus pallidus interneus (GPi). Thus, abnormally controlled and released DA from exogenous levodopa in the striatum provokes changes mainly in the "direct" striato-pallidal projection leading to decreased inhibitory output from basal ganglia neurons, leading to unrestrained thalamocortical drive, and the appearance of dyskinesias (Obeso et al. 2002). However, GPi hypoactivity cannot be the sole cause of development of dyskinesias in view of the fact that pallidotomy abolishes dyskinesias (Lozano 2000), and abnormal firing patterns in basal ganglia output neurons also play a role (Obeso et al. 2002). Indeed, abnormal patterns of neuronal activity, particularly in terms of enhanced synchronization of subthalamic nucleus activity in the theta/alpha band has been associated with both "peak-dose" and diphasic dyskinesias (Alonso-Frech et al. 2006; Alegre et al. 2012).

Generally, most severe motor complications as judged by complex "*on-off*" fluctuations and continuous and debilitating dyskinesias have virtually disappeared from the clinical scenario. This is probably due to a better and more rational use of dopaminergic drugs, the reduction in daily levodopa utilization and earlier treatment of motor complications with infusion therapies and surgery.

5 Nonmotor Features in PD

5.1 Cognitive Dysfunction

Cognitive dysfunction is common in PD, in particular in advanced stages. About 75–80 % of PD patients will develop dementia after 20 years of evolution. Cognitive impairment is clinically characterized by a progressive dysexecutive syndrome with attention deficit, and short-term memory impairment (Svenningsson et al. 2012). This is often accompanied by high sensitivity to develop psychotic manifestations in response to dopaminergic drugs.

It is now realized that already in earlier phase of the disease, about 25 % of nondemented PD patients suffer mild cognitive impairment (MCI), mostly in the executive domain. Clinicopathological studies demonstrated that dementia is associated with bradykinetic onset and higher cortical Lewy body burden which is combined with nondopaminergic mechanisms, i.e., cholinergic deficiency in the cortex (due to degeneration of ascending projections from the nucleus basalis of Meynert) (Paulus and Jellinger 1991; Bohnen and Albin 2009). Concomitant Alzheimer's disease-like change and cerebrovascular disease have also been proposed as possible substrates of PD dementia (Pagonabarraga and Kulisevsky 2012). In fact, it has been proposed that development of dementia in PD is better explained by the coincidence of Alzheimer's disease-like pathology *and* cortical Lewy bodies rather than by the presence of either of these alone (Compta et al. 2011) due to synergistic effects of amyloid, Tau, and Lewy bodies during neurodegeneration (Pagonabarraga and Kulisevsky 2012).

Depression affects 30–50 % of individuals with PD, with prevalences rates of 17 % for major depression, 22 % for minor depression, and 13 % for dysthymia. Apathy has an estimated prevalence of up to 60 % in PD (Aarsland et al. 2012). Lewy body deposition in brainstem areas including in serotoninergic raphe nuclei, in the noradrenergic locus coeruleus and in cholinergic limbic pathways are proposed structures underlying depression in PD (Frisina et al. 2009; Brooks and Pavese 2011; Remy et al. 2005). Using functional imaging, function of the serotonergic system has been investigated in PD using both PET (1C-DASB to depict brain serotonin transporter availability (Politis et al. 2010); and 11C-WAY 100635 PET as a marker of serotonin 5-HT1A function) and SPECT (123I-beta-CIT) ligands. While widespread loss of brain serotonergic innervation is a feature of advanced PD (Gutteman et al. 2007), it has been proposed that serotoninergic loss may not be overly relevant to the development of depression in PD (Brooks and Pavese 2011). Using 11C-RT132 as PET tracer that binds with similar affinity to both DA and noradrenaline membrane transporters the locus coeruleus and areas of the limbic system were identified to have decreased binding and these findings are probably independent from Lewy body pathology affecting the locus coeruleus (Brooks and Pavese 2011).

5.2 Sleep

Sixty to ninty eight percentage of PD patients encounter nocturnal sleep disturbances; in about 33 % of moderate to severe degree (Garcia-Borreguero et al. 2003; Diederich and McIntyre 2012). This includes nocturnal awakenings or sleep fragmentation, apnea, restless legs syndrome (RLS), periodic limb movement during sleep (PLMS), and REM sleep behavior disorder (RBD) (Raggi et al. 2013). The latter is characterized by vigorous movements during REM sleep without atonia and the occurrence of dream enactment. The RBD is present in 25–50 % of PD patients. It has been hypothesized that this may be caused by synucleinopathic involvement of the locus coeruleus, lower raphe, and/or pendunculopontine nucleus (with cholinergic denervation of the thalamus, as implicated in RBD) (Wolters 2009). The involvement of these structures early in the pathological staging may explain why many patients report of sleep abnormalities prior to motor symptom onset. Notably, presence of RBD in non-PD individuals is associated with a considerable risk of up to 80 % of developing PD or other defined neurodegenerative synucleinopathy (like dementia with Lewy bodies or multiple system atrophy (MSA)) (Postuma 2014), and patients should be monitored accordingly.

5.3 Gastrointestinal Function

Gastrointestinal dysfunction is very common in PD (Barone et al. 2009) and may be one of the earliest manifestations, in some preceding motor symptom onset by decades (Fig. 2). It may manifest with drooling and dysphagia (Nobrega et al. 2008), delayed gastric emptying accompanied by sickness and vomiting, and lower gastrointestinal symptoms, due to a dysfunction of the enteric nervous system with impaired or loss of the peristaltic transport, manifesting with constipation (Edwards et al. 1992). Of these, constipation is seen in over 50 % of PD patients constituting the most frequent gastrointestinal problem in PD (Martinez-Martin et al. 2007). There is increasing frequency with advancing disease. Pathologically, both central and peripheral mechanisms contribute to the development of constipation (Wolters 2009). In this regard, it is interesting that numerous studies have demonstrated alpha-synuclein pathology in the enteric nervous system. Thus, biopsies of the gut have revealed Lewy bodies and Lewy neurites in the mucosa and submucosa with a rostrocaudal gradient with highest density in the esophagus compared to the rectum (Derkinderen et al. 2011; Pouclet et al. 2012). Notably, one study identified abnormalities even in samples obtained prior to the motor onset (Shannon et al. 2012) which supports the Braak hypothesis of spreading disease from the vagal nerve to the brain (see above). However, there is high variability in the methods used between these studies; and confirmation of results are needed (Schneider et al. in preparation).

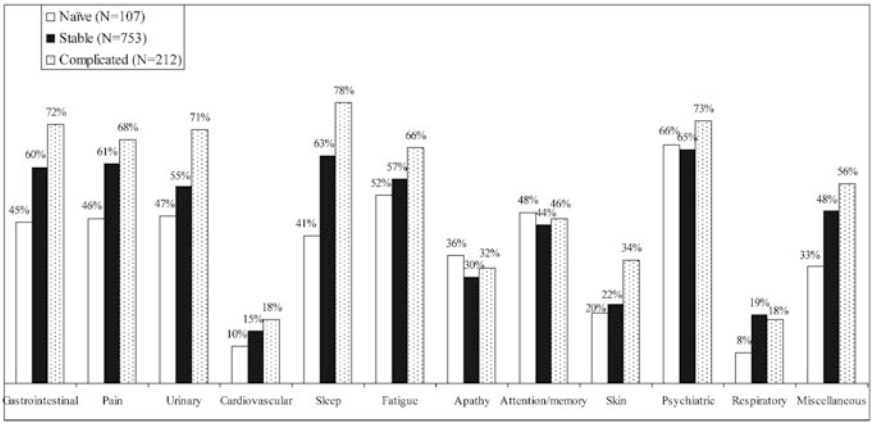

Fig. 2 Prevalence of nonmotor symptoms (NMS) in different stages of PD. The figure is taken from the Priamo Study (Barone et al. 2009) assessing different NMS (*gastrointestintal*, *urinary symptoms*, *pain*, *sleep disorders*, *skin changes* etc.) in a large cohort of 1072 patients. Prevalence of NMS domains according to patient's clinical status. Left (*white*) bar = drug-naive patients; middle (*black*) bar = patients with stable disease; right (*dotted*) bar = patients with PD in complicated disease phases

5.4 Urinary Tract Function and Sexuality

Urinary tract dysfunction is also a common problem in PD (Barone et al. 2009; Winge and Fowler 2006) (Fig. 2), albeit not as prominent as in the related disorder of MSA. Main symptoms comprise of storage dysfunction related to an overactive bladder contractility causing daytime frequency, urinary urgency and nocturia. Such storage problems are considered mainly the result of DA deficiency-induced disinhibition and hyperactivation of the micturation reflex, localized in the pontine storage and pontine micturition center in the brainstem (Wolters 2009; Yeo et al. 2012).

Sexual dysfunction in PD may be present with increased (hypersexuality) or decreased libido, or erectile dysfunction. Hypothalamic dysfunction is mostly responsible for the sexual dysfunction (decrease in libido and erectile dysfunction) in PD, via altered dopamine-oxytocin pathways (Yeo et al. 2012; Sakakibara et al. 2010), whereas hypersexuality occurs in the context of therapy with DA agonists (reviewed in Vilas et al. 2012).

5.5 Disturbed Sense of Smell and Taste

Olfactory loss is present to some degree in 80–96 % of PD patients and often precedes motor symptom onset (Katzenschlager and Lees 2004; Doty 2012). Total anosmia, however, is rare, and some patients are not aware of the deficit until their sense of smell is formally tested. On the other hand, an intact olfaction in patients with parkinsonism few years into the disease indicates a nonidiopathic PD pathology and should prompt re-evaluation. Abnormality of taste (diminished preception/threshold, taste identification, altered taste, or burning mouth) may also be present in PD (present in about 25 % of patients) (Kim et al. 2011; Kashihara et al. 2011; Shah et al. 2009).

Pathological studies demonstrated Lewy body pathology in the olfactory bulb and the primary olfactory cortex, present from early disease stages conforming to the Braak classification (Wolters 2009).

6 Differential Diagnosis

As mentioned above, the diagnosis of PD is made clinically. Blood tests and other investigations mainly aim at excluding secondary causes (Berardelli et al. 2013). For example, anatomical imaging may reveal vascular parkinsonism, hydrocephalus, or structural lesions of the basal ganglia. Functional imaging includes dopamine transporter (DAT) imaging to assess the integrity of the dopaminergic system or presence of presynaptic neuronal degeneration. Striatal uptake of the ligands for DAT (FP-CIT, beta-CIT, IPT, TRODAT) correlates with disease

severity, in particular bradykinesia and rigidity, and DAT imaging has been used as marker of progression in clinical trials. Notably, uptake is altered even from early disease phases; however, DA loss (reflected by an abnormal DAT scan) is also found in related forms of neurodegenerative parkinsonism (the so-called atypical forms of parkinsonism, e.g., MSA, supranuclear palsy, etc.). In some scenarios but not used in daily clinical routine, the use of other forms of functional imaging (such as MIBG cardiac scintigraphy) may provide further information. Blood tests help to exclude metabolic conditions (such as Wilson's disease). In young-onset cases genetic testing can ascertain or exclude some of the known monogenic forms.

7 Disease Progression, Survival Rate, and Causes of Death

Progressive neuronal loss and clinical deterioration are an inexorable process in PD. Progression of global disability in PD is attributed both to an increasing motor burden (mainly driven by poorly L-dopa-responsive motor symptoms, like postural instability, freezing of gait and falls, as well as by the evolution of levodopa-related motor complications) and to nonmotor (mainly cognitive) decline (Maetzler et al. 2009; Poewe 2009). Two general observations have been made. First, age at onset plays a pivotal role for progression. Studies suggest that the disease progresses more slowly in patients with young-onset PD, which may be due to age as the main factor because, with increasing age, these young-onset patients will more and more resemble classic older onset PD (van Rooden et al. 2010). The second observation concerns the clinical phentotype: While it is impossible to predict the progression rate on an individual basis, tremor-dominant PD tends to progress more slowly than the akinetic-rigid variant (Lewis et al. 2005; Sato et al. 2006; Selikhova et al. 2009). Akinetic-rigid PD seems to be associated with higher rates of cognitive decline and shorter survival rates.

In late disease stages (with survival for 15–20 years), about half of the patients require home care nursing due to the high prevalence of dementia or other severe disability. Survival time in PD has also been assessed in several studies. The Sidney multicenter study, for example, in the recent report on 20 year follow-up data, showed that median time to death was 12.4 years (Hely et al. 2008) After 20 years of follow-up, 74 % of the original patient cohort had died. Cognitive decline with dementia and older age at onset were identified as predictors of reduced survival time. It has been suggested that mortality rates increase with disease duration: thus, the standardized mortality ratio (that is the ratio of deaths in the PD group in relation to expected deaths in the general population) increased steadily in the Sydney Study from 1.8 at 5 years to 2.3 by year 10, and 2.7 by year 15; and eventually rose to 3.1 at 20 years disease duration compared to the general population (Hely et al. 2008). Main causes of death in PD include respiratory infection or insufficiency, cardiac failure but also cancer, and other causes (Roos et al. 1996; Sato et al. 2006).

8 Conclusions and Perspectives

It is current understanding that the pathological basis of motor PD is a depletion of nigrostriatal axon terminals, due to neuronal loss in the SNpc (with presence of Lewy bodies in the residual neurons). Involvement of adjacent areas and increasingly wide regions accounts for both nonmotor symptoms and motor features that occur in the advanced stages of PD and respond poorly to DA replacement therapy. They may reflect the fact that not only dopaminergic but other aminergic cell groups are also affected by PD pathology. Notably though, the Braak hypothesis remains contentious, particularly because the relationship between Lewy pathology and neuronal loss is quite unclear in most regions (Halliday et al. 2012; Jellinger 2012; Burke et al. 2008)

Most recently, there has been interest in possible pre-symptomatic diagnosis through investigations of noncerebral regions (such as the GI tract); but the validity of these novel approaches remains to be confirmed in future studies. Current therapy of PD remains symptomatic, but, hopefully, a better understanding of the underlying pathophysiology will lay the ground for disease-modifying treatments in the near future.

References

Aarsland D, Pahlhagen S, Ballard CG, Ehrt U, Svenningsson P (2012) Depression in Parkinson disease–epidemiology, mechanisms and management. Nat Rev Neurol 8(1):35–47

Alegre M, López-Azcárate J, Alonso-Frech F, Rodríguez-Oroz MC, Valencia M, Guridi J, Artieda J, Obeso JA (2012) Subthalamic activity during diphasic dyskinesias in Parkinson's disease. Mov Disord 27:1178–1181

Alonso-Frech F, Zamarbide I, Alegre M, Rodríguez-Oroz MC, Guridi J, Manrique M, Valencia M, Artieda J, Obeso JA (2006) Slow oscillatory activity and levodopa-induced dyskinesias in Parkinson's disease. Brain 129:1748–1757

Barone P, Antonini A, Colosimo C, Marconi R, Morgante L, Avarello TP, et al, PRIAMO study group (2009) The PRIAMO study: a multicenter assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease. Mov Disord 24(11):1641–1649

Beavan MS, Schapira AH (2013) Glucocerebrosidase mutations and the pathogenesis of Parkinson disease. Ann Med 45(8):511–521

Berardelli A, Wenning GK, Antonini A, Berg D, Bloem BR, Bonifati V et al (2013) EFNS/MDS-ES recommendations for the diagnosis of Parkinson's disease. Eur J Neurol 20(1):16–34

Bohnen NI, Albin RL (2009) Cholinergic denervation occurs early in Parkinson disease. Neurology 73:256–257

Bonifati V (2012) Autosomal recessive Parkinsonism. Parkinsonism Relat Disord 18(Suppl 1):S4–S6

Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24(2):197–211

Brooks DJ, Pavese N (2011) Imaging biomarkers in Parkinson's disease. Prog Neurobiol 95(4):614–628

Burke RE, Dauer WT, Vonsattel JP (2008) A critical evaluation of the Braak staging scheme for Parkinson's disease. Ann Neurol 64(5):485–491

Cenci MA, Lundblad M (2006) Post- versus presynaptic plasticity in L-DOPA-induced dyskinesia. J Neurochem 99(2):381–392
Cenci MA, Ohlin KE, Odin P (2011) Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease. CNS Neurol Disord Drug Targets 10(6):670–684
Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, Lashley T, Kallis C, Williams DR, de Silva R, Lees AJ, Revesz T (2011) Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain 134:1493–1505
Derkinderen P, Rouaud T, Lebouvier T, Bruley des Varannes S, Neunlist M, De Giorgio R (2011) Parkinson disease: the enteric nervous system spills its guts. Neurology 77(19):1761–1767
Diederich NJ, McIntyre DJ (2012) Sleep disorders in Parkinson's disease: many causes, few therapeutic options. J Neurol Sci 314(1–2):12–19
Donaldson I, Marsden CD, Schneider S, Bhatia K (2012) Marsden's book of movement disorders. Oxford University Press, Oxford
Dorsey ER, Constantinescu R, Thompson JP, Biglan K, Holloway RG, Kieburtz K, Marshall FJ, Ravina B, Schifitto G, Siderowf A, Tanner C (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 68:384–386
Doty RL (2012) Olfaction in Parkinson's disease and related disorders. Neurobiol Dis 46(3):527–552
Edwards LL, Quigley EM, Pfeiffer RF (1992) Gastrointestinal dysfunction in Parkinson's disease: frequency and pathophysiology. Neurology 42(4):726–732
Fearnley JM, Lees AJ (1991) Ageing Parkinson's disease: Substantia nigra regional selectivity. Brain 114(Pt 5):2283–2301
Frisina PG, Haroutunian V, Libow LS (2009) The neuropathological basis for depression in Parkinson's disease. Parkinsonism Relat Disord 15(2):144–148
Garcia-Borreguero D, Larrosa O, Bravo M (2003) Parkinson's disease and sleep. Sleep Med Rev 7(2):115–129
Gasser T (2010) Identifying PD-causing genes and genetic susceptibility factors: current approaches and future prospects. Prog Brain Res 183:3–20
Guttman M, Boileau I, Warsh J, Saint-Cyr JA, Ginovart N, McCluskey T, Houle S, Wilson A, Mundo E, Rusjan P, Meyer J, Kish SJ (2007) Brain serotonin transporter binding in non-depressed patients with Parkinson's disease. Eur J Neurol 14:523–528
Halliday G, McCann H, Shepherd C (2012) Evaluation of the Braak hypothesis: how far can it explain the pathogenesis of Parkinson's disease? Expert Rev Neurother 12(6):673–686
Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG (2008) The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord 23(6):837–844
Jellinger KA (2012) Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts. Mov Disord 27(1):8–30
Kashihara K, Hanaoka A, Imamura T (2011) Frequency and characteristics of taste impairment in patients with Parkinson's disease: results of a clinical interview. Int Med 50(20):2311–2315
Katzenschlager R, Lees AJ (2004) Olfaction and Parkinson's syndromes: its role in differential diagnosis. Curr Opin Neurol 17(4):417–423
Kim HJ, Jeon BS, Lee JY, Cho YJ, Hong KS, Cho JY (2011) Taste function in patients with Parkinson disease. J Neurol 258(6):1076–1079
Kordower JH, Olanow CW, Dodiya HB, Chu Y, Beach TG, Adler CH, Halliday GM, Bartus RT (2013) Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain 136:2419–2431
Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease. Lancet 373(9680):2055–2066
Lewis SJ, Foltynie T, Blackwell AD, Robbins TW, Owen AM, Barker RA (2005) Heterogeneity of Parkinson's disease in the early clinical stages using a data driven approach. J Neurol Neurosurg Psychiatry 76:343–348

Litvan I, Bhatia KP, Burn DJ, Goetz CG, Lang AE, McKeith I et al (2003) Movement disorders society scientific issues committee. Movement disorders society scientific issues committee report: SIC task force appraisal of clinical diagnostic criteria for Parkinsonian disorders. Mov Disord 18(5):467–486

Löhle M, Storch A, Reichmann H (2009) Beyond tremor and rigidity: non-motor features of Parkinson's disease. J Neural Transm 116(11):1483–1492

Lozano AM (2000) Neuronal recordings in Parkinson's disease patients with dyskinesias induced by apomorphine. Ann Neurol 47(1):141–146

Maetzler W, Liepelt I, Berg D (2009) Progression of Parkinson's disease in the clinical phase: potential markers. Lancet Neurol 8(12):1158–1171

Manson A, Stirpe P, Schrag A (2012) Levodopa-induced-dyskinesias clinical features, incidence, risk factors, management and impact on quality of life. J Parkinsons Dis 2(3):189–198

Martinez-Martin P, Schapira AH, Stocchi F, Sethi K, Odin P, MacPhee G, Brown RG, Naidu Y, Clayton L, Abe K, Tsuboi Y, MacMahon D, Barone P, Rabey M, Bonuccelli U, Forbes A, Breen K, Tluk S, Olanow CW, Thomas S, Rye D, Hand A, Williams AJ, Ondo W, Chaudhuri KR (2007) Prevalence of nonmotor symptoms in Parkinson's disease in an international setting; study using nonmotor symptoms questionnaire in 545 patients. Mov Disord 22(11):1623–1629

Nobrega AC, Rodrigues B, Torres AC, Scarpel RD, Neves CA, Melo A (2008) Is drooling secondary to a swallowing disorder in patients with Parkinson's disease? Parkinsonism Relat Disord 14(3):243–245

Obeso JA, Rodríguez-Oroz MC, Rodríguez M, Arbizu J, Giménez-Amaya JM (2002) The basal ganglia and disorders of movement: pathophysiological mechanisms. News Physiol Sci 17:51–55

Olanow WC, Kieburtz K, Rascol O, Poewe W, Schapira AH, Emre M, Nissinen H, Leinonen M, Stocchi F, Stalevo Reduction in Dyskinesia Evaluation in Parkinson's Disease (STRIDE-PD) Investigators (2013) Factors predictive of the development of Levodopa-induced dyskinesia and wearing-off in Parkinson's disease. Mov Disord 28(8):1064–1071

Ondo (2011) Motor complications in Parkinson's disease. Int J Neurosci 121:37–44

Pagonabarraga J, Kulisevsky J (2012) Cognitive impairment and dementia in Parkinson's disease. Neurobiol Dis 46(3):590–596

Pan-Montojo F, Schwarz M, Winkler C, Arnhold M, O'Sullivan GA, Pal A, Said J, Marsico G, Verbavatz JM, Rodrigo-Angulo M, Gille G, Funk RH, Reichmann H (2012) Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. Sci Rep 2:898

Paulus W, Jellinger K (1991) The neuropathologic basis of different clinical subgroups of Parkinson's disease. J Neuropathol Exp Neurol 50(6):743–755

Poewe W (2009) Clinical measures of progression in Parkinson's disease. Mov Disord 24(2):S671–S676

Politis M, Wu K, Loane C, Turkheimer FE, Molloy S, Brooks DJ, Piccini P (2010) Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. Neurology 75(21):1920–1927

Postuma RB (2014) Prodromal Parkinson's disease–using REM sleep behavior disorder as a window. Parkinsonism Relat Disord 20(Suppl 1):S1–S4

Pouclet H, Lebouvier T, Coron E, des Varannes SB, Rouaud T, Roy M, Neunlist M, Derkinderen P (2012) A comparison between rectal and colonic biopsies to detect Lewy pathology in Parkinson's disease. Neurobiol Dis. 45(1):305–309

Raggi A, Bella R, Pennisi G, Neri W, Ferri R (2013) Sleep disorders in Parkinson's disease: a narrative review of the literature. Rev Neurosci 24(3):279–291

Remy P, Doder M, Lees A, Turjanski N, Brooks D (2005) Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. Brain 128(Pt 6):1314–1322

Roos RA, Jongen JC, van der Velde EA (1996) Clinical course of patients with idiopathic Parkinson's disease. Mov Disord 11(3):236–242

Sakakibara R, Uchiyama T, Yamanishi T, Kishi M (2010) Genitourinary dysfunction in Parkinson's disease. Mov Disord 25(1):2–12

Sato K, Hatano T, Yamashiro K, Kagohashi M, Nishioka K, Izawa N et al, Juntendo Parkinson Study Group (2006) Prognosis of Parkinson's disease: time to stage III, IV, V, and to motor fluctuations. Mov Disord 21(9):1384–1395

Schneider SA, Alexoudi A, Böttner M, et al Can we use peripheral tissue biopsies to diagnose Parkinsońs disease? A review of the literature (in preparation)

Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ (2009) A clinico-pathological study of subtypes in Parkinson's disease. Brain 132:2947–2957

Shah M, Deeb J, Fernando M, Noyce A, Visentin E, Findley LJ, Hawkes CH (2009) Abnormality of taste and smell in Parkinson's disease. Parkinsonism Relat Disord 15(3):232–237

Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH (2012) Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases.Mov Disord 27(6):716–719

Sidransky E, Lopez G (2012) The link between the GBA gene and parkinsonism. Lancet Neurol 11(11):986–998

Stamey W, Davidson A, Jankovic J (2008) Shoulder pain: a presenting symptom of Parkinson disease. J Clin Rheumatol 14(4):253–254

Surmeier DJ, Schumacker PT (2013) Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. J Biol Chem 288(15):10736–10741

Svenningsson P, Westman E, Ballard C, Aarsland D (2012) Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment. Lancet Neurol 11(8):697–707

Thanvi B, Lo N, Robinson T (2007) Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 83(980):384–388

Truong DD, Wolters EC (2009) Recognition and management of Parkinson's disease during the premotor (prodromal) phase. Expert Rev Neurother 9(6):847–857

van Rooden SM, Heiser WJ, Kok JN, Verbaan D, van Hilten JJ, Marinus J (2010) The identification of Parkinson's disease subtypes using cluster analysis: a systematic review. Mov Disord 25(8):969–978

Vilas D, Pont-Sunyer C, Tolosa E (2012) Impulse control disorders in Parkinson's disease. Parkinsonism Relat Disord 18(Suppl 1):S80–S84

Visanji NP, Brooks PL, Hazrati LN, Lang AE (2013) The prion hypothesis in Parkinson's disease: braak to the future. Acta Neuropathol Commun 1(1):2

Voon V, Fernagut PO, Wickens J, Baunez C, Rodriguez M, Pavon N, Juncos JL, Obeso JA, Bezard E (2009) Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. Lancet Neurol 8:1140–1149

Winge K, Fowler CJ (2006) Bladder dysfunction in Parkinsonism: mechanisms, prevalence, symptoms, and management. Mov Disord 21(6):737–745

Wolters ECh (2009) Non-motor extranigral signs and symptoms in Parkinson's disease. Parkinsonism Relat Disord 15(Suppl 3):S6–S12

Wolters ECh, Braak H (2006) Parkinson's disease: premotor clinico-pathological correlations. J Neural Transm Suppl 70:309–319

Schneider SA, Alexoudi A, Boettner M, Deuschl G, Wedel T (in prep) Can we use peripheral tissue biopsies to diagnose Parkinsońs disease? A review of the literature

Yeo L, Singh R, Gundeti M, Barua JM, Masood J (2012) Urinary tract dysfunction in Parkinson's disease: a review. Int Urol Nephrol 44(2):415–424

Symptomatic Models of Parkinson's Disease and L-DOPA-Induced Dyskinesia in Non-human Primates

Tom M. Johnston and Susan H. Fox

Abstract Models of Parkinson's disease (PD) can be produced in several non-human primate (NHP) species by applying neurotoxic lesions to the nigrostriatal dopamine pathway. The most commonly used neurotoxin is MPTP, a compound accidentally discovered as a contaminant of street drugs. Compared to other neurotoxins, MPTP has the advantage of crossing the blood–brain barrier and can thus be administered systemically. MPTP-lesioned NHPs exhibit the main core clinical features of PD. When treated with L-DOPA, these NHP models develop involuntary movements resembling the phenomenology of human dyskinesias. In old-world NHP species (macaques, baboons), choreic and dystonic dyskinesias can be readily distinguished and quantified with specific rating scales. More recently, certain non-motor symptoms relevant to human PD have been described in L-DOPA-treated MPTP-NHPs, including a range of neuropsychiatric abnormalities and sleep disturbances. The main shortcomings of MPTP-NHP models consist in a lack of progression of the underlying neurodegenerative lesion, along with an inability to model the intracellular protein-inclusion pathology typical of PD. The strength of MPTP-NHP models lies in their face and predictive validity for symptomatic treatments of parkinsonian motor features. Indeed, these models have been instrumental to the development of several medical and surgical approaches that are currently applied to treat PD.

Keywords 6-hydroxydopamine · MPTP · Non-human primate · Dyskinesia · Non-motor

T.M. Johnston
Toronto Western Research Institute, University of Toronto, Toronto Western Hospital, 399 Bathurst St, Toronto, ON M5T 2S8, Canada

S.H. Fox (✉)
Division of Neurology, University of Toronto, Toronto Western Hospital, 399 Bathurst St, Toronto, ON M5T 2S8, Canada
e-mail: sfox@uhnresearch.ca

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1 Non-human Primate Models of PD

1.1 Introduction

Non-human primates (NHPs) have been used to generate the most robust and clinically useful models of parkinsonian symptoms. Models have been used to evaluate motor features, drug-induced side effects and, more recently, a variety of non-motor symptoms experienced in PD. Historically, prior models using NHPs were abandoned with the discovery of MPTP. Earlier models included systemic administration of the cholinergic agonists, carbachol and harmaline that induced a short-lived tremor (Everett et al. 1956), or electrolytic lesions of the midbrain, resulting in longer-lasting hypokinesia and tremor (Poirier 1960). However, neither of these models was selective for dopaminergic neurons. The neurotoxin, 6-hydroxydopamine (6-OHDA), was also tried in NHPs, either into medial forebrain bundle (Apicella et al. 1990; Annett et al. 1995) or striatum (Eslamboli 2005) and produced a stable hypokinesia. However, bilateral models resulted in profound akinesia that required intensive care of the animals (Mitchell et al. 1985). The discovery of the selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al. 1984) allowed the development of the gold standard NHP model of PD with stable, bilateral clinical features closely resembling idiopathic PD (Crossman et al. 1985; DeLong et al. 1985; Jenner et al. 1984). The MPTP model has been used for several decades now and has led to many of the pharmacological and surgical therapies that are currently used to treat PD (Aziz et al. 1991; Bergman et al. 1990; Brotchie 2005; Gomez-Mancilla and Bedard 1993; Jenner 2003a).

2 MPTP-Induced Parkinsonism

The neurochemical pathology of dopamine cell loss in MPTP-parkinsonism mimics human PD. Thus, MPTP induces a marked (>90 %) selective dopamine cell loss within the substantia nigra pars compacta (SNC). The pattern of cell loss in the SNC is similar to human PD with a ventro-lateral predominance (Burns et al. 1983; Gibb et al. 1987). However, dopamine loss occurs in the striatum in a uniform pattern, rather than the preferential loss in the putamen seen in human PD (Perez-Otano et al. 1994). There is also >50 % reduction in striatal spine density especially in sensorimotor post-commissural putamen and caudate (Villalba et al. 2009). Extra-nigral dopaminergic systems can be mildly affected, including cortex and limbic regions (Perez-Otano et al. 1991; Mitchell et al. 1985; Rose et al. 1989). Serotonergic and noradrenergic systems can be also affected by MPTP. Reduced 5-HT levels occur in the cingulate and frontal cortex and possibly striatum (Perez-Otano et al. 1991; Russ et al. 1991). The locus coeruleus has been reported to show degeneration in the MPTP macaque (Mitchell et al. 1985; Forno et al. 1986) with reduction in noradrenaline in the frontal cortex (Alexander et al. 1992; Pifl et al. 1991) and thalamus (Pifl et al. 2013). These pathological features induced by MPTP have been suggested to facilitate the development of drug-induced side effects and non-motor symptoms that also depend on non-dopaminergic systems.

The MPTP-primate, however, does not model the major pathophysiological hallmark of idiopathic PD, i.e. Lewy bodies. Increased intraneuronal alpha synuclein immunoreactivity has been reported within the SNC; however, this is not in the usual structural form of a Lewy body (Kowall et al. 2000). There is an effect of age on the level of this alpha synuclein in NHPs, with older animals having higher levels, similar to human PD (Chu and Kordower 2007). The absence of Lewy bodies in MPTP-NHPs has been suggested to relate to the relatively short-time post-MPTP that pathological studies are performed; however, a recent study in 2 animals confirmed no Lewy bodies even 10 years post-MPTP (Halliday et al. 2009). Thus, studying alpha synuclein pathology and neurodegeneration is not appropriate in the NHP and other models are primarily used for these indications (see review by Hatami and Chesselet 2014).

3 Generation of the MPTP Model of Advanced PD

The species of NHP used to generate models of PD depends on availability and costs. Old World species are superior in terms of modelling phenomenology of clinical features but are more costly. These include macaques, both rhesus (*sp mulatta*) and cymologous (*sp fascicularis*) (Burns et al. 1983; Mitchel et al. 1985); African green monkeys (Taylor et al. 1997) and baboons (Todd et al. 1996). Alternatively, New World species including common marmosets (Jenner et al. 1984) and squirrel monkeys (Langston et al. 1984) have the advantages of smaller size with

cheaper costs and easier husbandry but less articulate motor symptoms. Regimens of MPTP administration can be altered according to the type of model required (reviewed in Fox and Brotchie 2010). Thus, stability of the model, degree of dopamine cell loss, i.e. early versus advanced disease, and unilateral or bilateral models can be generated. The most common method is repeated systemic MPTP administration (e.g. 1–2 mg/kg) titrated over several days to months until a desired level of stable parkinsonism is achieved (Burns et al. 1983; Visanji et al. 2009b). Recovery may occur in some animals after a few months, and additional MPTP may be required in some to maintain the severity of symptomatology. Older animals are also more susceptible to MPTP, requiring lower doses (Ovadia et al. 1995). Alternative schedules have been tried to reduce recovery rates such as lower doses (0.2 mg/kg) over 2–3 weeks (Bezard et al. 1997; Meissner et al. 2003). Less advanced disease stages have been modelled using shorter treatments, e.g. 1 mg/kg for 3 days will generate partial lesions, with approximately 60 % tyrosine hydroxylase cell loss (Iravani et al. 2005). Such models are more suitable to evaluate neuroprotection strategies rather than symptomatic therapies. A unilateral model of intracarotid infusion of MPTP is now rarely used (Bankiewicz et al. 1986). Such treatment schedule results in less motor features and may not consistently respond to levodopa or develop dyskinesia, possibly due to compensatory mechanisms via crossover dopaminergic fibres (Lieu et al. 2011). Practical issues related to MPTP use and safety have been extensively reviewed elsewhere (Przedborski et al. 2001; Emborg 2007; Visanji and Brotchie 2005).

Stabilisation of the model is important. Following MPTP administration, animals need to be left for several (8–12) weeks, to prevent acute effects of MPTP affecting motor evaluations. At this stage, animals will exhibit motor symptoms reminiscent of advanced, untreated PD. To generate a model of advanced *treated* PD, levodopa is then initiated, usually 10–30 mg/kg (once or twice a day) dosing schedule. There is inter-animal variability in doses required. Levodopa (with a peripheral decarboxylase inhibitor to increase brain availability of dopamine) can be administered via oral gavage or subcutaneously. More predictable absorption is obtained if the animal has not eaten, due to competition with dietary amino acids to cross the blood–brain barrier. Over a period of several weeks, averaging 3–12w, levodopa-induced dyskinesia (LID) will gradually develop until a stable, reproducible phenotype is reached. There is species variability in development of LID such that squirrel monkeys tend to be most sensitive. As in human PD, the dose and duration of levodopa therapy (Smith et al. 2003) correlate with speed of onset of dyskinesia as well as severity of parkinsonism (Schneider et al. 2003). The time course of developing LID is much shorter than in human PD due to marked loss of striatal dopamine (>90 %), and some animals may develop dyskinesia on receiving the first dose of levodopa (Di Monte et al. 2000; Schneider 1989).

3.1 The Motor Symptoms of MPTP-Parkinsonism

The MPTP-primate exhibits the cardinal feature of PD, bradykinesia, which is manifest in the NHP by a global slowness of movement. Severely affected animals often sit motionless for long periods. Milder affected animals may move slowly or sit with reduced head checking movements and reduced overall spontaneous exploratory activity. Reduced facial movements including reduced blink rate occur. In addition, there is reduced speed of finer motor skills, e.g. picking up food/toys. Gait is also affected and animals will walk slowly. A particular parkinsonian phenomenon, 'freezing of gait', is common with animals exhibiting an inability to move for a few seconds, as if stuck in one place (Revuelta et al. 2012). Falls are rare due to parkinsonian disability per se and are more likely in the context of severe levodopa-induced dyskinesia (see below). The animals will sit with their neck and trunk flexed in a typical parkinsonian hunched posture. The classical 4–6 Hz resting tremor of PD is rare but has been described in the African green monkeys (Bergman et al. 1998). More commonly, a postural tremor may be seen when an animal is walking and reaching for objects. Freezing of gait may be accompanied by leg tremor at slightly higher than typical parkinsonian frequency (~7 Hz)) (Revuelta et al. 2012). Rigidity is present but is less commonly assessed as part of the rating scales as animals will need to be handled and thus disturbed. Methods to overcome this issue include use of a primate chair that enables the animal to be gently restrained and muscle tone evaluated.

Levodopa reverses all the motor symptoms induced by MPTP, thus modelling the response of PD patients. The anti-parkinsonian response is dose related, with higher levodopa doses produce quicker and longer-lasting response. Also in keeping with PD patients is that the first treatment with levodopa or dopamine agonist may not fully reverse symptoms and animals need a few days of treatment to develop a maximal response. The influence of the peripheral pharmacokinetics of levodopa is also important. Thus, absorption of levodopa is greatly enhanced if animals are not fed prior to administration of levodopa either oral or subcutaneous due to the competition of dietary amino acids for GI and blood–brain barrier amino acid transporters as per human PD.

There are many scales that have been developed to measure MPTP-parkinsonism. All are based on subjective clinical evaluations in a similar way to rating scales used to asses PD patients, such as the Unified Parkinson's disease rating scale (UPDRS). Thus, the NHP scales consist of assessment of severity, and possibly disability, of range of movement, bradykinesia, posture, alertness and tremor. Rigidity is less commonly included. Several rating scales have been published, for example (Gomez-Ramirez et al. 2006; Visanji et al. 2009b; Fox et al. 2012) (Table 1). Objective measures of total, or global motor activity, have been proposed using, e.g. activity monitors or using video analysis systems with accelerometers (Togasaki et al. 2005; Liu et al. 2009; Campos-Romo et al. 2009). These approaches can provide a useful additional objective measure for overall level of motor activity. However, such systems are unable to distinguish well between movement

Table 1 MPTP-NHP rating scale for parkinsonism

1	Range of movement score
	0 = walking on the floor and/or climbing on the walls or roof of the cage
	1 = walking on the floor of the cage only
	2 = movement of limbs and/or trunk, without locomotion
	3 = movement of head only, without locomotion
	4 = no movement
2	Bradykinesia score
	0 = normal speed and initiation of movement
	1 = mild slowing of movement
	2 = moderate slowing, difficulty initiating and maintaining movement, freezing
	3 = marked slowing, or unable to move, with prolonged freezing episodes
3	Postural abnormality score
	0 = normal, upright, holds head up, normal balance
	1 = hunched body, holds head up
	3 = hunched body and neck, face down, may lose balance
4	Checking behaviour
	0 = present, looking around, observant
	1 = absent

Method
Ratings are made for 5 min every 10–20 min, for the entire period of observation. The score given is representative of each 5-min observation period
Scores for each of the four listed components are summated to give a total parkinsonian disability score for each time point. (Johnston et al. 2010; Fox et al. 2012)

due to reversal of parkinsonism and increased movement due to dyskinesia. Clinical observation remains the gold standard to fully evaluate motor features of parkinsonism, in particular the presence of bradykinesia.

3.2 Levodopa-Induced Dyskinesia

One of the commonest uses of the MPTP-primate is in the evaluation of pharmacological therapies for levodopa-induced dyskinesia (LID) (Brotchie 2005; Fox et al. 2006a; Kalia et al. 2013). In a similar way to PD patients, LID develops progressively over the course of levodopa treatment until a stable response is reached (Clarke et al. 1987; Jenner 2003b; (Pearce et al. 1995; Visanji et al. 2006, 2009a).). The onset of LID usually takes between 2–12 weeks after start of levodopa therapy, depending on dose, species and degree of parkinsonism. Occasionally, LID can occur with first dose of levodopa. The phenomenology of LID is similar to human PD and is a mixture of hyperkinetic movements. Chorea is a rapid flowing movement of limbs and trunk, while dystonia consists of more sustained posturing of limbs, trunk, neck and tail, although often with movement, especially

in New Work species, sometimes referred to as 'mobile dystonia'. Old World species have less overall motor activity and exhibit dyskinesia more easily distinguishable as either chorea or dystonia (Boyce et al. 1990a, b), and dystonia is often the commonest dyskinesia. New World primates are overall more active, and often chorea and dystonia may be difficult to distinguish unequivocally (Henry et al. 1999; Pearce et al. 1995). LID is most commonly present when the levels of levodopa are maximal, so-called peak-dose dyskinesia (Fox et al. 2001). However, PD patients with dyskinesia can also experience dyskinesia at the onset and end of a dose of levodopa termed 'diphasic dyskinesia' and often experience dystonia in the off state (Obeso et al. 2000). Such phenomena are rarely described in non-human primates (Boyce et al. 1990b).

In keeping with human PD, we have shown that on early exposure to levodopa, there is a dose response reversal of parkinsonism with associated dyskinesia, whereas following chronic levodopa, this changes to a shorter latency to reversal of PD ('switch-on') and an all-or-none response with no increase in dyskinesia severity with increased levodopa doses (Mestre et al. 2010). Chronic levodopa treatment does not exacerbate the severity of dyskinesia, but decreases the minimal dose required to elicit dyskinesia. As such, once primed, it became essentially impossible to alleviate parkinsonism in a manner that was not compromised by dyskinesia, in keeping with advanced PD.

3.3 Levodopa-Induced Motor Fluctuations

PD patients frequently report a waning of benefit of their levodopa dose with a re-emergence of parkinsonian symptoms called 'wearing-off' (Nutt et al. 2002). This occurs over time and with chronic levodopa treatment and often accompanies LID. MPTP-primate also exhibit such wearing-off or variations in time to reversal of symptoms such as 'delayed ON' or No ON (Fox et al. 2010; Jenner 2003a). Animals can also exhibit what is termed 'beginning and end-of-dose worsening', in a similar way to PD subjects (Quinn 1998). Thus, following levodopa, there is a transient worsening of motor function before improvement, and then, as the beneficial response to levodopa is declining, there is a rebound worsening of parkinsonism to below-baseline values (Kuoppamaki et al. 2002).

In a similar way to MPTP-parkinsonism, there are many scales for motor complications used by a number of centres, including Monkey Dyskinesia Rating Scale (Pearce et al. 1995); Disability Scale for MPTP-treated primates (Fox et al. 2001); Monkey Quality of ON-time (Johnston et al. 2010); Modification of AIMS (Gomez and Mancilla 1993; Blanchet et al. 1995); Global Non-Human primate Dyskinesia rating Scale (Petzinger et al. 2001); and St Kitts Biomedical Primate Dyskinesia Scale (Taylor et al. 1990). The issues related to these scales and a proposed new scale were recently reviewed in Fox et al. (2012) (Table 2).

Table 2 MPTP-NHP dyskinesia disability rating scale

0 = Absent	
No abnormal movements	
Able to successfully perform at all of the following motor tasks	
	a. Climbing walls of cage or branch
	b. Locomoting on floor
	c. Eating fruit (changed from among, apple, kiwi fruit, orange, banana each day, randomly)
	d. Taking treat paste, e.g. peanut butter from inside Kong toy
	e. Playing with toys
1 = Mild	
Abnormal flicking movements of distal limbs and/or transient sustained limb contractions. Able to successfully perform all motor tasks. Abnormal movements are transient and intermittent; present for less than 30 % of the observation period	
2 = Moderate	
Abnormal flicking movements of distal limbs and/or transient sustained limb contractions. Able to successfully perform all motor tasks. Abnormal movements may be intermittent or continuous; present for more than 30 % of the observation period	
3 = Marked	
Abnormal flowing movements of limbs and/or sustained posture of leg, arm, trunk or neck. Unable to eat, take treat from Kong toys; animals may still be able to walk and climb, although limited by dyskinesia. Abnormal movements may be intermittent or continuous. Present less than 70 % of the observation period	
4 = Severe	
Abnormal flowing movements of limbs/sustained posture of leg, arm, trunk or neck.	
Dyskinesia interferes with ability to perform any motor tasks. Walking markedly limited and not able to climb. Abnormal movements are continuous; present more than 70 % of the observation period	

Method
Ratings are made for 5 min every 10–20 min, for the entire period of observation. The score given is representative of each 5-min observation period. (Johnston et al. 2010; Fox et al. 2012)

3.3.1 Primate Models of Parkinsonian Gait and Balance Dysfunction

Several motor symptoms of PD may become resistant to levodopa therapy with advancing disease, in particular gait and balance. Finding better therapeutic options for these symptoms is increasingly important. The pedunculopontine nucleus (PPN) has been implicated in gait, and recent studies have investigated the role of this nucleus in gait dysfunction in PD. Unilateral and bilateral lesions of the PPN in normal NHPs can induce bradykinesia (Nandi et al. 2008). In the MPTP-primate model, low frequency (5–10 Hz) stimulation of the PPN reversed akinesia independently of levodopa (Nandi et al. 2008). Other researchers have recently reported a model more in keeping with advanced PD using young macaques that combined a classical bilateral dopaminergic MPTP-lesion with a unilateral PPN cholinergic lesioning using urotensin, to induce a levodopa-responsive akinesia but

dopamine-unresponsive gait disorder. The clinical features included a flexed trunk deviated towards the side contralateral to the lesion and erect tail, increased knee angle and height of the pelvis, and persistent balance deficits that induced falls. The symptoms were maximal immediately after the lesion, with slight regression over 3–4 weeks but without returning to baseline (Grabli et al. 2013). Quantification of gait and postural abnormalities was performed with the animals walking in a hallway after a training period and parameters measured include speed and length of steps, back curve, knee angle, height of the pelvis and tail position. Balance deficit was rated between 0 and 3 based on the frequency of disequilibrium in the hallways (number of disequilibrium occurring during 10 min, averaged on five sessions) (Karachi et al. 2010). Such a model, if replicated, may be useful in evaluating potential therapies for gait and balance in PD.

3.3.2 Non-motor Complications of Advancing PD

Several non-motor symptoms have been modelled using the MPTP-NHP model. These include drug-induced neuropsychiatric behaviours, cognitive impairment and sleep disorders.

3.4 Neuropsychiatric Behaviours

Neuropsychiatric symptoms in PD patients include behavioural disturbances, such as hallucinations and paranoid delusions, psychomotor agitation, impulse control disorders and complex motor stereotypies (called 'punding') (Voon and Fox 2007). These symptoms are both due to disease-related pathology, as well as side effects of dopaminergic medications. MPTP-NHPs treated with high doses of dopaminergic drugs will often exhibit abnormal repetitive, exaggerated and driven gross motor behaviours which are distinct from dyskinesia and parkinsonism and may represent behavioural correlates of neural processes of these neuropsychiatric symptoms in PD. Such behaviours have been noted by investigators in the past, e.g. agitation (Pearce et al. 1995), climbing behaviour (Boyce et al. 1990b), 'hallucinatory-like behaviour' (Blanchet et al. 1998) and hyperactivity (Akai et al. 1995). We have recently validated a psychosis-like behaviour rating scale for measuring these behaviours as a means of evaluating potential side effects of drug therapies (Visanji et al. 2006; Fox et al. 2006b, 2010). The scale consists of four behavioural categories: hyperkinesia (fast movements), response to non-apparent stimuli (possible hallucinatory-like behaviours), repetitive grooming (representing compulsive activity) and stereotypies (including pacing, repetitive side-to-side jumping and running in circles) (Rating scale and video reviewed in Visanji et al. 2006).

3.5 Cognitive Impairment

Cognitive problems are common in PD and range from mild cognitive impairment to dementia. The pattern of cognitive loss includes memory, visuospatial skills and fronto-striatal cognitive deficits. In the MPTP-NHP, chronic deficits in executive and attentional tasks (including delayed response, delayed matching to sample, visual discrimination and object retrieval/detour tasks) have been demonstrated, even in animals with minimal motor deficits (Pessiglione et al. 2004; Schneider and Kovelowski 1990; Taylor et al. 1990; Vezoli et al. 2011). Treatment with levodopa does not reverse these findings and may worsen cognitive problems (Decamp and Schneider 2009). The MPTP-primate thus shows promise as a model of cognitive deficits in PD; however, none of the currently used agents for cognitive problems in PD, such as acetylcholinesterase inhibitors, have been evaluated to date.

3.6 Sleep Disorders

Sleep disturbances are common in PD patients and are due to medications as well as disease pathology that results in disturbance of the sleep–wake cycle with insomnia and excessive daytime sleepiness. In addition, REM sleep behaviour disorder (RBD) is an early, preclinical symptom that can predate motor signs (Postuma et al. 2009). The MPTP-NHP also experiences sleep disturbances. In an MPTP-marmoset model of untreated early PD (low-dose MPTP with mild motor symptoms), high-amplitude EMG bouts during REM sleep relative to control animals were demonstrated suggestive of RBD (Verhave et al. 2011). Other studies in MPTP-lesioned-treated rhesus monkeys using long-term continuous electroencephalographic monitoring via implanted miniaturized telemetry device have shown progressive sleep deterioration, fragmentation and reduced sleep efficacy with increased sleepiness during the day by about 50 %; however, no RBD was shown (Barraud et al. 2009). A recent study reported differential effects of dopamine D1 and D2 agonists on sleep architecture in MPTP-NHPs, suggesting use in evaluating therapeutics for PD (Hyacinthe et al. 2014).

4 Concluding Remarks

The MPTP-NHP remains the most useful symptomatic model of advanced PD. The major weaknesses of the model lie in its inability to provide a truly progressive neurodegenerative process and to replicate the accumulation of misfolded alpha synuclein in intracellular inclusion such as Lewy bodies. However, for the purpose of parkinsonian motor symptoms, L-DOPA-induced dyskinesia and certain non-motor complications that occur in advanced PD the MPTP-NHP remains the gold standard with respect to the translational process.

References

Akai T, Ozawa M, Yamaguchi M, Mizuta E, Kuno S (1995) Combination treatment of the partial D2 agonist terguride with the D1 agonist SKF 82958 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned parkinsonian cynomolgus monkeys. J Pharmacol Exp Ther 273:14–309

Alexander GM, Schwartzman RJ, Brainard L, Gordon SW, Grothusen JR (1992) Changes in brain catecholamines and dopamine uptake sites at different stages of MPTP parkinsonism in monkeys. Brain Res 588:9–261

Annett LE, Torres EM, Ridley RM, Baker HF, Dunnett SB (1995) A comparison of the behavioural effects of embryonic nigral grafts in the caudate nucleus and in the putamen of marmosets with unilateral 6-OHDA lesions. Exp Brain Res 103:71–355

Apicella P, Trouche E, Nieoullon A, Legallet E, Dusticier N (1990) Motor impairments and neurochemical changes after unilateral 6-hydroxydopamine lesion of the nigrostriatal dopaminergic system in monkeys. Neuroscience 38:66–655

Aziz TZ, Peggs D, Sambrook MA, Crossman AR (1991) Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the primate. Mov Disord 6:92–288

Bankiewicz KS, Oldfield EH, Chiueh CC, Doppman JL, Jacobowitz DM, Kopin IJ (1986) Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Life Sci 39:7–16

Barraud Q, Lambrecq V, Forni C, McGuire S, Hill M, Bioulac B, Balzamo E, Bezard E, Tison F, Ghorayeb I (2009) Sleep disorders in Parkinson's disease: the contribution of the MPTP non-human primate model. Exp Neurol 219:82–574

Bergman H, Wichmann T, DeLong MR (1990) Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 249:8–1436

Bergman H, Raz A, Feingold A, Nini A, Nelken I, Hansel D, Ben-Pazi H, Reches A (1998) Physiology of MPTP tremor. Mov Disord 13(Suppl 3):29–34

Bezard E, Imbert C, Deloire X, Bioulac B, Gross CE (1997) A chronic MPTP model reproducing the slow evolution of Parkinson's disease: evolution of motor symptoms in the monkey. Brain Res 766:12–107

Blanchet PJ, Calon F, Martel JC, Bédard PJ, Di Paolo T, Walters RR, Piercey MF (1995) Continuous administration decreases and pulsatile administration increases behavioral sensitivity to a novel dopamine D2 agonist (U-91356A) in MPTP-exposed monkeys. J Pharmacol Exp Ther 272(2):9–854

Blanchet PJ, Konitsiotis S, Chase TN (1998) Amantadine reduces levodopa-induced dyskinesias in parkinsonian monkeys. Mov Disord 13:798–802

Boyce S, Clarke CE, Luquin R, Peggs D, Robertson RG, Mitchell IJ, Sambrook MA, Crossman AR (1990a) Induction of chorea and dystonia in parkinsonian primates. Mov Disord 5:3–7

Boyce S, Rupniak NM, Steventon MJ, Iversen SD (1990b) Characterisation of dyskinesias induced by L-dopa in MPTP-treated squirrel monkeys. Psychopharmacology 102:7–21

Brotchie JM (2005) Nondopaminergic mechanisms in levodopa-induced dyskinesia. Mov Disord 20:31–919

Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci U S A 80:50–4546

Campos-Romo A, Ojeda-Flores R, Moreno-Briseno P, Fernandez-Ruiz J (2009) Quantitative evaluation of MPTP-treated nonhuman parkinsonian primates in the HALLWAY task. J Neurosci Methods 177:8–361

Chu Y, Kordower JH (2007) Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: Is this the target for Parkinson's disease? Neurobiol Dis 25:49–134

Clarke CE, Sambrook MA, Mitchell IJ, Crossman AR (1987) Levodopa-induced dyskinesia and response fluctuations in primates rendered parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). J Neurol Sci 78:80–273

Crossman AR, Mitchell IJ, Sambrook MA (1985) Regional brain uptake of 2-deoxyglucose in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. Neuropharmacology 24:91–587

Decamp E, Schneider JS (2009) Interaction between nicotinic and dopaminergic therapies on cognition in a chronic Parkinson model. Brain Res 1262:109–114. http://www.ncbi.nlm.nih.gov/pubmed/19368843

DeLong MR, Crutcher MD, Georgopoulos AP (1985) Primate globus pallidus and subthalamic nucleus: functional organization. J Neurophysiol 53:43–530

Di Monte DA, McCormack A, Petzinger G, Janson AM, Quik M, Langston WJ (2000) Relationship among nigrostriatal denervation, parkinsonism, and dyskinesias in the MPTP primate model. Mov Disord 15(3):66–459

Emborg ME (2007) Nonhuman primate models of Parkinson's disease. ILAR J 48:55–339

Eslamboli A (2005) Marmoset monkey models of Parkinson's disease: which model, when and why? Brain Res Bull 68:9–140

Everett GM, Blockus LE, Shepperd IM (1956) Tremor induced by tremorine and its antagonism by anti-Parkinson drugs. Science 79:124

Forno LS, Langston JW, DeLanney LE, Irwin I, Ricaurte GA (1986) Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. Ann Neurol 20:55–449

Fox SH, Brotchie JM (2010) The MPTP lesioned non-human primate models of PD; Past, present and future. Prog Brain Research 184:133–157

Fox SH, Henry B, Hill MP, Peggs D, Crossman AR, Brotchie JM (2001) Neural mechanisms underlying peak-dose dyskinesia induced by levodopa and apomorphine are distinct: evidence from the effects of the alpha(2) adrenoceptor antagonist idazoxan. Mov Disord 16:50–642

Fox SH, Lang AE, Brotchie JM (2006a) Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: keys to success and roads to failure. Mov Disord 21:94–1578

Fox SH, Visanji NP, Johnston TH, Gomez-Ramirez J, Voon V, Brotchie JM (2006b) Dopamine receptor agonists and levodopa and inducing psychosis-like behavior in the MPTP primate model of Parkinson disease. Arch Neurol 63:4–1343

Fox SH, Visanji NP, Reyes G, Huot P, Gomez-Ramirez J, Johnston TH, Brotchie JM (2010) Development of psychosis-like behaviors and motor complications with de novo levodopa treatment in the MPTP primate model of Parkinson's disease. Can J Neurol Sci 37:86–95

Fox SH, Johnston TH, Li Q, Brotchie J, Bezard E (2012) A critique of available scales and presentation of the Non-Human Primate Dyskinesia Rating Scale. Mov Disord 27(11):8–1373

Gibb WR, Lees AJ, Wells FR, Barnard RO, Jenner P, Marsden CD (1987) Pathology of MPTP in the marmoset. Adv Neurol 45:90–187

Gomez-Mancilla B, Bedard PJ (1993) Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. Clin Neuropharmacol 16:27–418

Gomez-Ramirez J, Johnston TH, Visanji NP, Fox SH, Brotchie JM (2006) Histamine H3 receptor agonists reduce L-dopa-induced chorea, but not dystonia, in the MPTP-lesioned nonhuman primate model of Parkinson's disease. Mov Disord 21:46–839

Grabli D, Karachi C, Folgoas E, Monfort M, Tande D, Clark S, Civelli O, Hirsch EC, Francois C (2013) Gait disorders in parkinsonian monkeys with pedunculopontine nucleus lesions: a tale of two systems. J Neurosci 33:93–11986

Halliday G, Herrero MT, Murphy K, McCann H, Ros-Bernal F, Barcia C, Mori H, Blesa FJ, Obeso JA (2009) No Lewy pathology in monkeys with over 10 years of severe MPTP Parkinsonism. Mov Disord 24:23–1519

Hatami A, Chesselet MF (2014) Transgenic rodent models to study alpha-synuclein pathogenesis with a focus on cognitive deficits, *Current Topics in Behavioural Neuroscience*, In press

Henry B, Fox SH, Peggs D, Crossman AR, Brotchie JM (1999) The alpha2-adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian actions of L-dopa in the MPTP-lesioned primate model of Parkinson's disease. Mov Disord 14:53–744

Hyacinthe C, Imbert C, Barraud Q, Tison F, Bezard E, Ghorayeb I (2014) D1 receptor agonist improves sleep-wake parameters in experimental parkinsonism. Neurobiol Dis 63:4–20

Iravani MM, Syed E, Jackson MJ, Johnston LC, Smith LA, Jenner P (2005) A modified MPTP treatment regime produces reproducible partial nigrostriatal lesions in common marmosets. Eur J Neurosci 21:54–841

Jenner P (2003a) The contribution of the MPTP-treated primate model to the development of new treatment strategies for Parkinson's disease. Parkinsonism Relat Disord 9:7–131

Jenner P (2003b) The MPTP-treated primate as a model of motor complications in PD: primate model of motor complications. Neurology 61:S4–11

Jenner P, Rupniak NM, Rose S, Kelly E, Kilpatrick G, Lees A, Marsden CD (1984) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. Neurosci Lett 50:85–90

Johnston TH, Savola JM, Fox SH, Brotchie JM (2010) The α_2 adrenergic antagonist fipamezole prolongs the anti-parkinsonian actions of L-DOPA in the MPTP-lesioned macaque. Mov Disord 25(13):93–2084

Kalia L, Brotchie JM, Fox SH (2013) Novel nondopaminergic targets for motor features of Parkinson's disease: Review of recent trials. Mov Disord 28:44–131

Karachi C, Grabli D, Bernard FA, Tande´ D, Wattiez N, Belaid H, Bardinet E,Prigent A, Nothacker HP, Hunot S, Hartmann A, Lehericy S, Hirsch EC, Francois C (2010) Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease. J Clin Invest 120:2745–2754

Kowall NW, Hantraye P, Brouillet E, Beal MF, McKee AC, Ferrante RJ (2000) MPTP induces alpha-synuclein aggregation in the substantia nigra of baboons. NeuroReport 11:3–211

Kuoppamaki M, Al-Barghouthy G, Jackson M, Smith L, Zeng BY, Quinn N, Jenner P (2002) Beginning-of-dose and rebound worsening in MPTP-treated common marmosets treated with levodopa. Mov Disord 17:7–1312

Langston JW, Langston EB, Irwin I (1984) MPTP-induced parkinsonism in human and non-human primates–clinical and experimental aspects. Acta Neurol Scand Suppl 100:49–54

Lieu CA, Deogaonkar M, Bakay RA (2011) Subramanian T Dyskinesias do not develop after chronic intermittent levodopa therapy in clinically hemiparkinsonian rhesus monkeys. Parkinsonism Relat Disord. 17(1):9–34

Liu N, Yue F, Tang WP, Chan P (2009) An objective measurement of locomotion behavior for hemiparkinsonian cynomolgus monkeys. J Neurosci Methods 183:94–188

Meissner W, Prunier C, Guilloteau D, Chalon S, Gross CE, Bezard E (2003) Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. Mol Neurobiol 28:18–209

Mestre TJT, Brotchie JM, Fox S (2010) Evolution of the "short duration" response to L-DOPA in the MPTP-lesioned non-human primate model of Parkinson's disease. Mov Disord 25(Suppl 2): S417

Mitchell IJ, Cross AJ, Sambrook MA, Crossman AR (1985) Sites of the neurotoxic action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the macaque monkey include the ventral tegmental area and the locus coeruleus. Neurosci Lett 61:195–200

Nandi D, Jenkinson N, Stein J, Aziz T (2008) The pedunculopontine nucleus in Parkinson's disease: primate studies. Br J Neurosurg 22, Suppl 1:S4–8

Nutt JG, Carter JH, Lea ES, Sexton GJ (2002) Evolution of the response to levodopa during the first 4 years of therapy. Ann Neurol 51:93–686

Obeso JA, Rodriguez-Oroz MC, Rodriguez M, DeLong MR, Olanow CW (2000) Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. Ann Neurol 47:S22-32, discussion S32-4

Ovadia A, Zhang Z, Gash DM (1995) Increased susceptibility to MPTP toxicity in middle-aged rhesus monkeys. Neurobiol Aging 16:7–931

Pearce RK, Jackson M, Smith L, Jenner P, Marsden CD (1995) Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (Callithrix Jacchus). Mov Disord 10:40–731

Perez-Otano I, Herrero MT, Oset C, De Ceballos ML, Luquin MR, Obeso JA, Del Rio J (1991) Extensive loss of brain dopamine and serotonin induced by chronic administration of MPTP in the marmoset. Brain Res 567:32–127

Perez-Otano I, Oset C, Luquin MR, Herrero MT, Obeso JA, Del Rio J (1994) MPTP-induced parkinsonism in primates: pattern of striatal dopamine loss following acute and chronic administration. Neurosci Lett 175:5–121

Pessiglione M, Guehl D, Jan C, Francois C, Hirsch EC, Feger J, Tremblay L (2004) Disruption of self-organized actions in monkeys with progressive MPTP-induced parkinsonism: II. Effects of reward preference. Eur J Neurosci 19:46–437

Petzinger GM, Quik M, Ivashina E, Jakowec MW, Jakubiak M, Di Monte D, Langston JW (2001) Reliability and validity of a new global dyskinesia rating scale in the MPTP-lesioned non-human primate. Mov Disord 16(2):7–202

Pifl C, Schingnitz G, Hornykiewicz O (1991) Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on the regional distribution of brain monoamines in the rhesus monkey. Neuroscience 44:591–605

Pifl C, Hornykiewicz O, Blesa J, Adánez R, Cavada C, Obeso JA (2013) Reduced noradrenaline, but not dopamine and serotonin in motor thalamus of the MPTP primate: relation to severity of parkinsonism. J Neurochem 125:62–657

Poirier LJ (1960) Experimental and histological study of midbrain dyskinesias. J Neurophysiol 23:51–534

Postuma RB, Gagnon JF, Vendette M, Fantini ML, Massicotte-Marquez J, Montplaisir J (2009) Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. Neurology 72:300–1296

Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M (2001) The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem 76:74–1265

Quinn NP (1998) Classification of fluctuations in patients with Parkinson's disease. Neurology 51: S25–9

Revuelta GJ, Uthayathas S, Wahlquist AE, Factor SA, Papa SM (2012) Non-human primate FOG develops with advanced parkinsonism induced by MPTP Treatment. Exp Neurol 237(2):9–464

Rose S, Nomoto M, Jenner P, Marsden CD (1989) Transient depletion of nucleus accumbens dopamine content may contribute to initial akinesia induced by MPTP in common marmosets. Biochem Pharmacol 38:81–3677

Russ H, Mihatsch W, Gerlach M, Riederer P, Przuntek H (1991) Neurochemical and behavioural features induced by chronic low dose treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the common marmoset: implications for Parkinson's disease? Neurosci Lett 123:8–115

Schneider JS (1989) Levodopa-induced dyskinesias in parkinsonian monkeys: relationship to extent of nigrostriatal damage. Pharmacol Biochem Behav 34(1):6–193

Schneider JS, Kovelowski CJ 2nd (1990) Chronic exposure to low doses of MPTP. I. Cognitive deficits in motor asymptomatic monkeys. Brain Res 519:8–122

Schneider JS, Gonczi H, Decamp E (2003) Development of levodopa-induced dyskinesias in parkinsonian monkeys may depend upon rate of symptom onset and/or duration of symptoms. Brain Res 990:38–44

Smith LA, Jackson MJ, Hansard MJ, Maratos E, Jenner P (2003) Effect of pulsatile administration of levodopa on dyskinesia induction in drug-naive MPTP-treated common marmosets: effect of dose, frequency of administration, and brain exposure. Mov Disord 18:95–487

Taylor JR, Elsworth JD, Roth RH, Sladek JR Jr, Redmond DE Jr (1990) Cognitive and motor deficits in the acquisition of an object retrieval/detour task in MPTP-treated monkeys. Brain 113(Pt 3):37–617

Taylor JR, Elsworth JD, Roth RH, Sladek JR Jr, Redmond DE Jr (1997) Severe long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the vervet monkey (Cercopithecus aethiops sabaeus). Neuroscience 81:55–745

Todd RD, Carl J, Harmon S, O'Malley KL, Perlmutter JS (1996) Dynamic changes in striatal dopamine D2 and D3 receptor protein and mRNA in response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) denervation in baboons. J Neurosci 16:82–7776

Togasaki DM, Hsu A, Samant M, Farzan B, DeLanney LE, Langston JW, Di Monte DA, Quik M (2005) The Webcam system: a simple, automated, computer-based video system for quantitative measurement of movement in nonhuman primates. J Neurosci Methods 145:66–159

Verhave PS, Jongsma MJ, Van den Berg RM, Vis JC, Vanwersch RA, Smit AB, Van Someren EJ, Philippens IH (2011) REM sleep behavior disorder in the marmoset MPTP model of early Parkinson disease. Sleep 34(8):25–1119

Vezoli J, Fifel K, Leviel V, Dehay C, Kennedy H, Cooper HM, Gronfier C, Procyk E (2011) Early presymptomatic and long-term changes of rest activity cycles and cognitive behavior in a MPTP-monkey model of Parkinson's disease. PLoS ONE 6(8):e23952

Villalba RM, Lee H, Smith Y (2009) Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. Exp Neurol 215:7–220

Visanji NP, Brotchie JM (2005) MPTP-induced models of Parkinson's disease in mice and non-human primates. Curr Protoc Pharmacol Chapter 5:Unit5.42

Visanji NP, Gomez-Ramirez J, Johnston TH, Pires D, Voon V, Brotchie JM, Fox SH (2006) Pharmacological characterization of psychosis-like behavior in the MPTP-lesioned nonhuman primate model of Parkinson's disease. Mov Disord 21:91–1879

Visanji NP, Fox SH, Johnston T, Reyes G, Millan MJ, Brotchie JM (2009a) Dopamine D3 receptor stimulation underlies the development of L-DOPA-induced dyskinesia in animal models of Parkinson's disease. Neurobiol Dis 35:92–184

Visanji NP, Fox SH, Johnston TH, Millan MJ, Brotchie JM (2009b) Alpha1-adrenoceptors mediate dihydroxyphenylalanine-induced activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaques. J Pharmacol Exp Ther 328:83–276

Voon V, Fox SH (2007) Medication-related impulse control and repetitive behaviors in Parkinson disease. Arch Neurol 64:96–1089

Neuroinflammation in Parkinson's Disease Animal Models: A Cell Stress Response or a Step in Neurodegeneration?

Carolina Cebrián, John D. Loike and David Sulzer

Abstract The motor symptoms of Parkinson's disease are due to the progressive degeneration of dopaminergic neurons in the substantia nigra. Multiple neuroinflammatory processes are exacerbated in Parkinson's disease, including glial-mediated reactions, increased expression of proinflammatory substances, and lymphocytic infiltration, particularly in the substantia nigra. Neuroinflammation is also implicated in the neurodegeneration and consequent behavioral symptoms of many Parkinson's disease animal models, although it is not clear whether these features emulate pathogenic steps in the genuine disorder or if some inflammatory features provide protective stress responses. Here, we compare and summarize findings on neuroinflammatory responses and effects on behavior in a wide range of toxin-based, inflammatory and genetic Parkinson's disease animal models.

Keywords Parkinson's disease · Neuroinflammation · Neurodegeneration · Animal models · Microglia · Proinflammatory cytokines · Lymphocytes

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C. Cebrián · D. Sulzer
Department of Neurology, Columbia University Medical Center,
New York, NY 10032, USA

J.D. Loike
Department of Physiology and Cellular Biophysics,
Columbia University Medical Center, New York, NY 10032, USA

D. Sulzer (✉)
Departments of Psychiatry and Pharmacology, Columbia University Medical Center,
New York, NY 10032, USA
e-mail: ds43@columbia.edu

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1 Introduction

Parkinson's disease (PD), the most common movement disorder (Lee et al. 2009), is characterized by a progressive loss of dopamine (DA) releasing neurons in the substantia nigra (SN) pars compacta (SNc), resulting in slowness of movement, rigidity, and tremor (Jankovic 2008), as well as the death of neurons in other catecholaminergic and cholinergic nuclei (Sulzer and Surmeier 2013). A central feature of PD is the presence of neuronal intracellular proteinaceous inclusions that contain alpha-synuclein (α-syn) known as Lewy bodies or Lewy neurites (Braak and Del Tredici 2010; Del Tredici and Braak 2012; Sekiyama et al. 2012).

Extensive microgliosis as a feature of the SNc of PD patients has been well documented since the initial report by Charles Foix in 1925 (Foix and Nicolesco 1925), who drew outstanding illustrations of activated microglia, which they labeled *neuroglia,* in PD brain, along with extracellular remnants of neuromelanin and Lewy bodies. This discovery was ignored for decades, in part as a peripheral immune response in the central nervous system (CNS) requires peripheral immune cells to traverse the blood-brain barrier (BBB), which was thought to be a rare event due to the "immune-privileged" nature of the brain (Engelhardt and Coisne 2011). This assumption was superseded as more recent studies described how cellular BBB permeability is regulated as a stress response (Franzén et al. 2003; Ransohoff and Perry 2009; Rezai-Zadeh et al. 2009).

The contemporary revival of interest in inflammatory processes associated with PD occurred when McGeer et al. (1988a, b) confirmed Foix's results by demonstrating activated microglia in the SN of patients postmortem. This topic has since become a major focus of PD research as covered in multiple reviews (Mena and García de Yébenes 2008; Tufekci et al. 2012; Blandini 2013; Sanchez-Guajardo et al. 2013a). Evidence supporting neuroinflammation in PD includes postmortem studies of brain, analyses of pro-inflammatory cytokines in serum and cerebrospinal fluid (CSF), PD risk factor associations with cytokine and major histocompatibility complex (MHC) class II (MHC-II) polymorphisms, and epidemiological studies of nonsteroidal anti-inflammatory use (Frank-Cannon et al. 2009; Hirsch and Hunot 2009; Lee et al. 2009; Tansey and Goldberg 2010). Whether neuroinflammation is a cause of PD pathogenesis or a secondary stress response remains, however, remains an elusive issue.

There is in our opinion no clear evidence that neuroinflammation is a primary trigger of PD, but there have long been hints that viral infection could be involved

in some cases, most famously a parkinsonism in patients who survived the Spanish flu outbreaks and von Economo's encephalitis during World War I (Henry et al. 2010). It has been suggested that systemic infection/inflammation exaggerates pathogenic events associated with PD and intensifies symptoms (Kortekaas et al. 2005; Collins et al. 2012) or exacerbates neuronal dysfunction during the prodromal stage (Lee et al. 2009). Another hypothesis is that stressed neurons activate microglia that release factors that further damage neurons (Frank-Cannon et al. 2009; Hirsch and Hunot 2009; Tansey and Goldberg 2010; Zhang et al. 2011a) causing a "vicious cycle" of neuroinflammation and neurodegeneration (Tansey and Goldberg 2010; Hoban et al. 2013). Such studies have led to exploration of anti-inflammatory therapies in PD, although to date these have shown limited success. It is important to emphasize that inflammatory steps are stress responses and may in some instances provide neuroprotection.

In this chapter, we review the literature on how animal models have been used to examine neuroinflammatory processes in PD. For each, we highlight the immune cells elicited, the inflammatory markers that are upregulated, the effects on neurodegeneration, and the behavioral manifestations that may be related to neuroinflammation. There is a very extensive and often contradictory literature on these responses, and as to our knowledge these have not been summarized in a single review, we hope that this chapter will assist the interpretation and design of future research. While evidence for neuroinflammation in most models is clear, the understanding of its role in pathogenesis, protection, and changes in behavior for the most part remains limited.

2 Neuroinflammation in Animal Models of PD

2.1 Toxin-based Models

2.1.1 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

This toxin appeared in an initial report of a chemistry graduate student who injected himself intravenously with a synthetic heroin substitute and exhibited a long-lasting motor disorder within a few days (Davis et al. 1979) due to MPTP as a impurity (Kopin 1987). Postmortem examinations after a second appearance of MPTP as an impurity in a synthetic opiate revealed the presence of activated microglia that apparently persisted years after drug exposure (Langston et al. 1999).

MPTP was adapted as a PD model in rodents and primates. These studies showed that this compound acts as a lipophilic protoxin that crosses the BBB and is converted to the toxic metabolite 1-methyl-4-phenylpyridinium (MPP^+) by astrocytes and serotonergic neurons (Riachi et al. 1989) by monoamine oxidase-B (Ransom et al. 1987). MPP^+ is then released into the extracellular space and accumulated by the DA transporter (DAT) into DA neurons, causing a bilateral degeneration of the nigrostriatal tract (Taylor et al. 2013). MPP^+ produces

neurodegeneration through the blockade of electron transport chain enzyme complexes I, III, and IV (Desai et al. 1996). Additional factors modulate MPTP toxicity including iron, expression of the vesicular monoamine transporter, reactive oxygen species (ROS), and apoptosis (Salama and Arias-Carrion 2011).

Cellular Response

In mice, MPTP induces a rapid microglial activation that peaks prior to the loss of the DA neurons (Czlonkowska et al. 1996), a response particularly severe in the SAMP8 mouse line that has been used as a model of senility (Liu et al. 2010). Activation of the adaptive immune system by MPTP is shown by increased lymphocyte infiltration (Kurkowska Jastrzebska et al. 1999), primarily by T cells (Hirsch and Hunot 2009). Consistently, results with T-cell-receptor (TCR)-β-chain-deficient, immunodeficient and RAG1 knockout mice demonstrate that T-cell-deficiency attenuates MPTP-induced neurodegeneration (Brochard et al. 2009; Reynolds et al. 2010). Mice-deficient for inflammatory genes, including apoptosis signal-regulating kinase 1 (Lee et al. 2012a) and dynorphin (Wang et al. 2012), show attenuated microglial and astrocyte activation and are protected from MPTP toxicity. It has been suggested that D3 DA receptors on CD4+ T cells regulate their response to MPTP (González et al. 2013).

In non-human primates, HLA-DR-reactive microglia are found in SN following MPTP administration (McGeer et al. 2003). This activation is triggered early and persists for at least 35 months (Vázquez-Claverie et al. 2009).

Cytokine Response

Mice lacking gamma interferon (IFN-γ) or tumor necrosis factor-alpha (TNF-α) receptors displayed attenuated MPTP degeneration (Mount et al. 2007; Sriram et al. 2002; Barcia et al. 2011). In mouse striatum and SN, MPTP increases expression of the cytokines interleukin (IL)-1-beta (IL-1β), IL-6, IL-7, IL-10, the cytokine receptors IL-1R, IL-3R, IL-4R, IL-10R, the inflammation-related transcription factor NFκB (Grünblatt et al. 2001), the chemokine receptor CXCR4 and the chemokine ligand CXCL12 (Shimoji et al. 2009). In CSF of C57BL/6 but not BALB/C mice, MPTP increased the cytokines IL-10, IL-12, IL-13, IFN-γ, TNF-α and the monocyte chemotactic protein 1 (MCP-1) (Yasuda et al. 2008): this differences may be because the peripheral immune system in the C57BL/6 strain is more prone to a proinflammatory Th1 (T helper 1) phenotype that produces IFN-γ, while Balb/c mice tend to mount an anti-inflammatory Th2 (T helper 2) response (Sanchez-Guajardo et al. 2013a).

In monkeys, MPTP activates IFN-γ and TNF-α in SN microglia and astrocytes, and IFN-γ receptor signaling is increased in SN glia (Barcia et al. 2011). Microarray analysis of MPTP treated monkeys confirmed an increased expression of inflammation-related genes, including IL-11, chemokines, and complement system genes (Ohnuki et al. 2010).

In Vivo and Behavioral Features Related to Inflammation

Most studies on neuroinflammation and motor symptoms induced by MPTP have been performed in mice (Chung et al. 2010; L'Episcopo et al. 2010; Gupta et al. 2011; Lee et al. 2012b; Wang et al. 2012; Roy et al. 2012; Esposito et al. 2012; Ghosh et al. 2012). Together, these studies indicate that drugs that block inflammation are protective against MPTP alterations in behavior, including the antidepressant paroxetine (Chung et al. 2010), nitric oxide-donating nonsteroidal anti-inflammatory drugs (L'Episcopo et al. 2010), selective cyclooxygenase (COX)-2-inhibitors (Gupta et al. 2011), sodium phenylbutyrate (Roy et al. 2012), the fatty acid amide palmitoylethanolamide (Esposito et al. 2012), the antioxidant diapocynin (Ghosh et al. 2012), the partial of N-methyl-D-aspartate (NMDA) receptor agonist D-cycloserine (Wang et al. 2010), and the anti-inflammatory aescin (Selvakumar et al. 2014).

In monkeys that received a single intracarotid arterial injection of MPTP, activation of the peroxisome proliferator-activated receptor gamma (PPAR-γ) attenuated neuroinflammation, as measured by decreased CD68+ cells. Monkeys treated with PPAR-γ also displayed improved motor skills (Swanson et al. 2011).

2.1.2 6-hydroxydopamine (6-OHDA)

6-OHDA is a widely used catecholaminergic neurotoxin (Bové and Perier 2012), which in contrast to the typical systemic administration of MPTP to mice, is usually delivered directly to the medial forebrain bundle, SNc, or the striatum by stereotaxy in rats (Fulceri et al. 2006).

DA neuronal degeneration by 6-OHDA is due to its uptake by DAT. 6-OHDA is readily oxidized in the cytosol to produce ROS, including hydrogen peroxide and its corresponding *p*-quinone (von Coelln et al. 2001; Mazzio et al. 2004), which produces oxidative stress and mitochondrial respiration dysfunction (Barnum and Tansey 2010). The toxin further decreases striatal glutathione and superoxide dismutase (Perumal et al. 1992; Kunikowska and Jenner 2001), increases iron in SN (Oestreicher et al. 1994), and directly inhibits electron transport (Glinka et al. 1997).

Cellular Response

6-OHDA causes neuroinflammatory responses (Schober 2004; Tufekcy et al. 2012; Taylor et al. 2013) including reactive astrocytosis (Gomide et al. 2005; Wachter et al. 2010) and microglial activation (Akiyama and McGeer 1989; Marinova-Mutafchieva et al. 2009; Cicchetti et al. 2002). The microglial activation can be blocked by minocycline (He et al. 2001) or the COX-2 inhibitor celecoxib, which can delay DA cell loss (Sanchez-Pernaute et al. 2004).

The inflammatory profile observed in 6-OHDA-lesioned animals can depend on timing and the site of injection. For example, when 6-OHDA was injected into the striatum, microglial activation was more robust in striatum than SN (Armentero et al. 2006). Na et al. (2010), however, suggest that this is time-dependent as intrastriatal 6-OHDA increased inflammatory gene expression in striatum 3 days after the injection, but SN showed a similar inflammatory response after a week.

Cytokine Response

6-OHDA activates inflammatory features including NF-κB-mediated responses accompanied by inhibition of antioxidant systems regulated by Nrf2 (Tobón-Velasco et al. 2013), TNF-α (Mogi et al. 2000) and complement component 1q subcomponent-binding protein (Park et al. 2010).

Neuroprotection from 6-OHDA is provided by inhibiting inflammation with a peroxisome proliferator-activated receptor gamma agonist (Sadeghian et al. 2012), silencing the enzyme inducible nitric oxide synthase (iNOS) (Li et al. 2012) or blocking TNF-α (McCoy et al. 2006; Harms et al. 2011; Pabon et al. 2011; Zhang et al. 2011b).

In Vivo and Behavioral Features Related to Inflammation

As 6-OHDA can be delivered into one side of the brain, it provides a means to measure effects of DA depletion by studying rotational behavior (Pycock 1980), often activated by amphetamine. Blockade of soluble TNF-α signaling in vivo provided neuroprotection to DA neurons from 6-OHDA-induced death and attenuated rotational behavior (McCoy et al. 2006).

NK1, a substance P receptor antagonist, administered immediately after 6-OHDA injection, protected DA neurons, preserved barrier integrity, reduced neuroinflammation, and significantly improved motor function (Thornton and Vink 2012). A CD200R-blocking antibody injected into striatum of rats treated with 6-OHDA showed a significant increase in contralateral rotation and a significant decrease in DA SN neurons with remarkably increased activation of microglia and proinflammatory cytokines (Zhang et al. 2011c).

Interestingly, human IL-10 gene transfer in rats unilaterally injected with 6-OHDA inhibited forelimb akinesia (Johnston et al. 2008). An antagonist of metabotropic receptor mGluR5 immediately ameliorated 6-OHDA-induced akinesia, although it did not modify neuronal survival or neuroinflammation (Ambrosi et al. 2010).

A recent study in mice reports that swimming is effective in attenuating behavior impairments and signs of inflammation including increased IL-1β levels (Goes et al. 2014) from 6-OHDA and alters glutathione peroxidase, glutathione reductase, and glutathione S-transferase activities. The authors conclude that protective effects induced by exercise on PD are due to the induction of antioxidant and anti-inflammatory responses.

2.1.3 Rotenone and Paraquat

Exposure to the herbicide paraquat and the pesticide rotenone is linked to an increased risk of PD in epidemiological studies (Kamel et al. 2007; Baldereschi et al. 2008; Hancock et al. 2008), and both have been adapted for PD models. Rotenone is highly lipophilic and crosses the BBB to diffuse into neurons where it inhibits complex I of the mitochondrial respiratory chain and causes neurodegeneration of SN neurons; however, reports of its selectivity for neurotoxicity are variable (Betarbet et al. 2000; Cicchetti et al. 2009). Paraquat is a charged molecule that does not cross the BBB and requires a neutral amino acid transporter for neuronal accumulation (Shimizu et al. 2001). In the cytosol, paraquat generates superoxide and impairs recycling of oxidized glutathione.

Cellular and Cytokine Responses

Rotenone toxicity is linked to increased ROS production with oxidative damage in the midbrain and striatum and microglial activation (OX-42 immunoreactivity) in SN and striatum with minimal astrocytosis (Sherer et al. 2003a, b, c) prior to DA cell loss. Microglia may mediate rotenone-induced neuronal degeneration through IFN-γ (Mount et al. 2007) or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-generated superoxide production (Gao et al. 2003). Inhibition of microglia by minocycline in vitro provides protection from rotenone (Casarejos et al. 2006). Rotenone does not directly activate microglia in vitro (Gao et al. 2002b, 2003; Shaikh and Nicholson 2009; Klintworth et al. 2009), and so its toxicity has been suggested to result from disturbing CD200R-CD200L microglia-DA neuron cross talk (Wang et al. 2011).

Intracerebral injection of paraquat induces loss of DA neurons (Liou et al. 1996), activates microglia (Purisai et al. 2007), elevates expression of TNF-α, IL-1β, and NF-κB (Yadav et al. 2012), iNOS (Cicchetti et al. 2005; Gupta et al. 2010), and produces α-syn-containing inclusion bodies (Manning-Bog et al. 2002). IFN-γ knockout mice injected with paraquat displayed reduced cell death and microglial activation, decreased proinflammatory enzymes (iNOS and COX-2) and cytokines (TNF-α, IL-1β), and increased trophic factors (Mangano et al. 2012). It has been suggested that elevation of cytosolic DA induced by paraquat participates in the neurotoxicity via quinone formation (Izumi et al. 2014).

In Vivo and Behavioral Features Related to Inflammation

Sindhu et al. (2005) observed that Sprague-Dawley rats infused intranigrally with rotenone exhibited spontaneous contralateral rotations immediately after recovery from anesthesia. In rats treated subcutaneously with rotenone, an adenosine triphosphate-sensitive potassium channel opener, iptakalim, normalized rotenone-induced

behavioral symptoms, degeneration of SNc DA neurons, microglial activation, and mRNA levels of TNF-α and COX-2 (Zhou et al. 2007a, 2008).

Intraperitoneal injection of paraquat in mice causes DA neurotoxicity in SN, frontal cortex and hippocampus, motor impairment assessed by curling and footprint tests, brain-specific ROS generation, and microgliosis with increased levels of TNF-α and IL-1β (Mitra et al. 2011).

2.2 Inflammatory Models

2.2.1 LPS (lipopolysaccharide)

LPS, an endotoxin in the outer membrane of Gram-negative bacteria, is widely used to trigger immune response in animals (Liu and Bing 2011). It activates Toll-like receptor (TLR) 4, which is highly expressed in microglia (Sanchez-Guajardo et al. 2013a). While no reports have indicated that septic individuals with high LPS develop PD (Fang et al. 2012), it has been used to initiate DA neuronal death and examine neuroinflammation in animal models (Lee et al. 2009).

In neuronal culture, DA neurons are twice as sensitive to LPS as non-DA neurons, and LPS toxicity occurs via microglial activation (Bronstein et al. 1995; Gayle et al. 2002; Dutta et al. 2008). In vivo, LPS inflammation also causes selective toxicity of DA neurons (Qin et al. 2007; Hunter et al. 2009; Machado et al. 2011; Tufekci et al. 2011) that apparently lack TLR4 receptor (Sanchez-Guajardo et al. 2013a).

Chronic low-dose intranigral LPS infusion in rats induced delayed, chronic, and progressive loss of DA SNc neurons (Gao et al. 2002a), while intrauterine exposure to LPS causes a degeneration of SNc neurons in offspring (Carvey et al. 2003). Chronic low-dose intraperitoneal LPS acted in concert with parkin deficiency to induce nigral DA neuron loss (Frank-Cannon et al. 2008), suggesting that a non-specific immunogenic stimulus in concert with a permissive genetic mutation could cause parkinsonism.

Cellular and Cytokine Responses

Intranigral LPS triggers activation of astrocytes and microglia; damages SN DA neurons (Castaño et al. 1998; Iravani et al. 2005; Qin et al. 2007; Pott Godoy et al. 2008), releases proinflammatory factors including IL-1α, TNF-α, IL-1β, and inducible nitric oxide synthase (iNOS, Qin et al. 2007; Tomás-Camardiel et al. 2004; Hernández-Romero et al. 2008), and increases COX-2 (de Meira Santos Lima et al. 2006; Geng et al. 2011), ROS and matrix metalloproteinase-3 (MMP-3) (Qin et al. 2004; McClain et al. 2009).

In aged mice, LPS caused progressive loss of DA neurons, whereas younger mice or aged mice treated with the anti-inflammatory nonsteroid drug HCT1026 were resistant (L'Episcopo et al. 2011). A sustained elevation of TNF-α has been observed in striatum and mesencephalon of rats prenatally exposed to LPS (Ling et al. 2004).

Several studies support a central role for TNF-α in LPS neurodegeneration. LPS-induced nigral DA damage is inhibited by dexamethasone or soluble TNF-α inhibitors (Castaño et al. 2002) or neutralizing antibodies against TNF-α or IL-1 that block microglial activation (Dutta et al. 2008; McCoy and Tansey 2008). An IL-1 receptor antagonist reduced LPS-induced TNF-α and IFN-γ release and loss of DA neurons (Koprich et al. 2008). LPS did not release IL-1β and NFκB p65, or kill SN neurons in mice deficient for TNF-α receptors (Qin et al. 2007).

A role for DA in response to LPS was indicated since alpha-methyl-p-tyrosine, an inhibitor of tyrosine hydroxylase (TH), prevented LPS-induced nigral DA death (De Pablos et al. 2005). The iron chelator desferrioxamine was also protective against LPS-induced SN neuronal degeneration (Zhang et al. 2012), consistent with a role for iron-mediated oxidative stress, which again may be related to DA oxidation or mitochondrial stress.

In Vivo and Behavioral Features Related to Inflammation

Similar to the 6-OHDA model above, four weeks of running exercise prior to LPS prevented the loss of DA neurons and motor dysfunction. Running did not change the LPS-induced status of microglia activation or the levels of cytokines/chemokines, but restored normal brain-derived neurotrophic factor (BDNF)-TrkB signaling. Blocking BDNF with a TrkB receptor antagonist abolished running's protective effect, while intrastriatal perfusion of BDNF alone blocked LPS-induced DA neuron loss (Wu et al. 2011).

Multiple in vivo studies confirm the role for TNF in LPS neurotoxicity that was suggested in cellular studies. Subacute LPS injection into SN induces PD-like symptoms in mice including aggregation of α-syn. Behavioral deficits were observed in wild-type (WT) and TNF-α knockout (KO) mice, but IL-1 KO mice behaved normally. TH gene expression was attenuated by LPS in WT and TNF-α KO, but not in IL-1 KO mice (Tanaka et al. 2013). Chronic in vivo co-infusion of a TNF blocker with LPS into the SNc of rats attenuated amphetamine-induced rotation, (McCoy et al. 2006), as did a glucagon-like peptide 1 receptor agonist (Harkavyi et al. 2008).

LPS injection in the striatum causes intracytoplasmic accumulation of α-syn and ubiquitin, defects in the mitochondrial respiratory chain, and extensive S-nitrosylation/nitration of mitochondrial complex I. The mitochondrial injury was prevented by treatment with L-N(6)-(l-iminoethyl)-lysine, an iNOS inhibitor. These results implicate neuroinflammation-induced S-nitrosylation/nitration of mitochondrial complex I in LPS-induced mitochondrial malfunction and degeneration of SN neurons (Choi et al. 2009). In another study, striatal LPS caused microgliosis

but not nigrostriatal neurodegeneration, with only a transient motor dysfunction (Hoban et al. 2013). The administration of a peptide antagonist of IL-1 signaling to rats after receiving LPS blocked inflammation as well as deficits in social activity and memory (Klementiev et al. 2014).

In mice, both neuroinflammation and motor impairment were exacerbated when LPS bilateral striatal injections were followed by the administration of methamphetamine (Jung et al. 2010), further indicating a role for DA itself in LPS damage.

2.2.2 Polyinosinic:polycytidylic Acid [poly(I:C)]

Introduction and Cellular and Cytokine Responses

Polyinosinic:polycytidylic acid [poly(I:C)] is structurally similar to double-stranded RNA and widely used to study antiviral responses such as production of type I interferons (Liu et al. 2012). Poly(I:C) is an agonist for TLR3, which is found in CNS microglia and is upregulated by LPS and IFN-γ (Olson and Miller 2004).

Single poly(I:C) injections to the rat SN do not cause SN neurodegeneration, but increase the susceptibility of DA neurons to a subsequent low dose of 6-OHDA (Deleidi et al. 2010). This induced long-lasting inflammation in SN and dorsolateral striatum involving microglia, astrocytes, and perivascular and parenchymal CD68+ macrophages and the chemokines, MCP-1, and RANTES (CCL5) in the SN, and IL-1β, IL-6, TNF-α, MCP-1, and transforming growth factor beta 1 (TGF-β1). IL-1R antagonist treatment rescued neurons from poly(I:C) and 6-OHDA toxicity (Deleidi et al. 2010).

In Vivo and Behavioral Features Related to Inflammation

In mice, pretreatment with poly(I:C) enhanced DA neuron loss in SN elicited by subsequent paraquat treatment. The neuronal loss was accompanied by robust signs of microglial activation, and increased expression of the catalytic subunit (gp91) of the NADPH oxidase oxidative stress enzyme. These findings suggest that viral agents can sensitize microglial-dependent inflammatory responses, rendering nigral DA neurons vulnerable to environmental toxin exposure (Bobyn et al. 2012).

2.2.3 Prostaglandin J2

Introduction and Cellular and Cytokine Responses

The major prostaglandin in mammalian brain is prostaglandin D2, which undergoes spontaneous dehydration to generate the bioactive cyclopentenone prostaglandins

of the J2 series. J2 prostaglandins are highly reactive and neurotoxic products of inflammation that impair ubiquitin/proteasome function and cause accumulation of ubiquitinated proteins (Li et al. 2004). A single report that we are aware of indicates that prostaglandin D2 administration into the SN activates microglia and astrocytes and produces neurodegeneration with α-syn aggregation (Pierre et al. 2009).

2.3 Genetic Models

2.3.1 α-Synuclein (SNCA)

Introduction

Over 20 loci and 15 disease-causing genes for parkinsonism have been identified (Deng and Yuan 2014). Mutations in seven genes are robustly associated with autosomal dominant (SNCA, LRRK2, EIF4G1, VPS35) or recessive (parkin/PARK2, PINK1, DJ1/PARK7) PD or parkinsonism. Of these, α-syn has received particular attention as aggregation of this protein is a neuropathological feature of the vast majority of PD cases. SNCA was also the first PD gene identified (Polymeropoulos et al. 1997), and encodes a 140 amino acid synaptic vesicle-associated protein that regulates synaptic vesicle exocytosis (Abeliovich et al. 2000; Fortin et al. 2005; Larsen et al. 2006; Nemani et al. 2010). Shortly thereafter, α-syn was identified as the main component of Lewy bodies and Lewy neurites in PD patients (Spillantini et al. 1997). At least five-point SNCA mutations have been linked to familial PD (Krüger et al. 1998; Zarranz et al. 2004) as well as gene duplications and triplication (Singleton et al. 2003; Ross et al. 2008), indicating that excessive levels of the normal protein also cause PD. SNCA triplication carriers display an approximately 10-year earlier onset and a more rapid disease course than duplication carriers, who more closely resemble patients with idiopathic PD (Kasten and Klein 2013).

α-syn is a natively unfolded soluble protein that can aggregate to form oligomers or protofibrils, and eventually insoluble polymers or fibrils (Conway et al. 2000a, b). The toxic contribution from different α-syn forms is a major area of investigation (Bungeroth et al. 2014). Both unfolded and aggregated might be neurotoxic via a variety of pathways including inhibition of protein degradation by chaperone-mediated autophagy (Cuervo et al. 2004) and membrane permeabilization (Rochet et al. 2004; Mosharov et al. 2006; Staal et al. 2008). Oligomeric α-syn may mediate neurodegeneration by disrupting synaptic vesicles (Rockenstein et al. 2014). α-syn fibrils, like prions, have been shown to seed new α-syn reactions (Miake et al. 2002; Masuda-Suzukake et al. 2013). Anti-aggregation compounds may help address these questions by inhibiting α-syn aggregate formation (Herva et al. 2014).

In animal studies, α-syn overexpression in combination with MPTP or rotenone increased neurodegeneration (Dauer et al. 2002; Mulcahy et al. 2013). LPS causes neuronal death in transgenic mice that express normal or mutant (A53T) α-syn

(Gao et al. 2008, 2011). Proteomic analysis of brains from mice overexpressing the human A30P mutation identified increased oxidized metabolic proteins (Poon et al. 2005), while mutant A53T mice displayed mitochondrial damage and degeneration (Martin et al. 2006).

An alternate model that emulates Lewy body pathology, synaptic dysfunction, and neuronal death in PD is to use fibrils generated from full-length and truncated recombinant α-syn (Volpicelli-Daley et al. 2011). In young asymptomatic α-syn transgenic mice, intracerebral injections of brain homogenates derived from older transgenic mice exhibiting α-syn pathology induced intracellular Lewy bodies/Lewy neurites-like inclusions and neurological symptoms (Luk et al. 2012a). In WT non-transgenic mice, a single intrastriatal inoculation of synthetic α-syn fibrils led to the cell-to-cell transmission of pathologic α-syn and PD-like Lewy pathology in anatomically interconnected regions in the SN but not in the adjacent ventral tegmental area, which is relatively spared in PD (Luk et al. 2012b). It is possible that this spread of pathology occurs via an inflammatory mechanism.

Cellular and Cytokine Responses

A number of SCNA mouse models (KO, overexpressors, and transgenics) have been generated (Chesselet 2008) although few exhibit DA cell loss (for exceptions, see Lin et al. 2012 and Janezic et al. 2013). Some of these models, however, display α-syn aggregation, gliosis, mitochondrial abnormalities, and functional abnormalities in the nigrostriatal system (Dawson et al. 2010).

The initial α-syn transgenic mouse introduced by Masliah et al. (2000) using a platelet-derived growth factor subunit B promoter exhibited human α-syn-immunoreactive inclusions most frequently seen in neurons in the deeper layers of the neocortex, the CA3 region of the hippocampus, olfactory bulb and occasionally in the SN. The mice also displayed lower TH^+ levels within the striatum than non-transgenic littermates. A transgenic mice harboring a SCNA transgene with a Thy1 promoter (Rockenstein et al. 2002) showed astroglial and microglial activation, elevated levels of TNF-α in the striatum, and increased levels of TNF-α, TLR1, TLR4, and TLR8 in the SN (Watson et al. 2012). Microglial activation and high levels of TNF-α were found in the SN of mice expressing human α-syn under control of the mouse TH promoter (Su et al. 2008). Expression of truncated human α-syn increased CD11b-positive microglia in the SN of transgenic mice (Tofaris et al. 2006). Transgenic mice that express A53T α-syn displayed astrogliosis in the spinal cord (Giasson et al. 2002).

It has been suggested that in α-syn transgenic models, microglia are not extensively activated unless the mice are treated with LPS or possess further genetic modifications (Sekiyama et al. 2012). Microgliosis was found in cortex and hippocampus of A30P overexpressing mice under the prion protein (PrP) promoter along with truncated and oligomeric α-syn (Gomez-Isla et al. 2003). α-syn pathological accumulation has been associated with microgliosis in the E46K α-syn transgenic under the PrP (Emmer et al. 2011). An A53T α-syn transgenic mouse

line under the PrP promoter and the A30P+A53T α-syn line under the TH promoter featured changes in microglia cell numbers and altered expression patterns in multiple genes related to the inflammatory responses (Lee et al. 2002; Miller et al. 2007).

Mice and DA neuronal cultures derived from mice with WT and mutant A53T α-syn in an α-syn-null background exposed to LPS, displayed neuroinflammation DA neuronal death and accumulation of cytoplasmic α-syn inclusions (Gao et al. 2008). Inhibition of nitric oxide and superoxide produced by microglia provided substantial neuroprotection.

To date, the few α-syn transgenic mouse models to display neuronal death are as follows: (1) a line of tetracycline-regulated inducible transgenic mice that overexpressed A53T α-syn in DA neurons; these developed motor disabilities, decreased DA release, fragmentation of Golgi apparatus, and impairment of autophagy/lysosome degradation pathways (Lin et al. 2012); (2) a line of bacterial artificial chromosome (BAC) mice overexpressing WT hSNCA140 generated by injection of BAC DNA containing WT hSNCA (Janezic et al. 2013).

In contrast to transgenic models, overexpression of α-syn by means of viral vectors has produced significant SN neuronal death (Ulusoy et al. 2010). In mice, overexpression of WT human α-syn can lead to slow degeneration of DA neurons (Theodore et al. 2008; Harms et al. 2013). Four weeks after injection, there was a marked increase in CD68-positive microglia and greater infiltration of B and T lymphocytes in the SNc of the rAAV2-SYN group than in controls. At 12 weeks, CD68 staining declined, but B- and T-cell infiltration persisted. Expression of proinflammatory cytokines was enhanced, whereas markers of alternative activation (i.e., arginase I, IL-4 and IL-13) were not altered. Increased immunoreactivity for mouse immunoglobulin was detected at all time points in the rAAV2-SYN animals. Thus, overexpression of α-syn is sufficient to trigger neuroinflammation featuring microglial activation and stimulation of adaptive immunity (Theodore et al. 2008). Overexpression of full-length human α-syn in mouse SNc induced extensive MHC-II expression by microglia, while mice lacking of MHC-II were protected from α-syn-induced microglial activation and DA neuron degeneration (Harms et al. 2013).

In rats, transgenic α-syn overexpression produced α-syn-positive cytoplasmic inclusions and swollen, dystrophic neurites selectively in the nigral DA neurons with a loss of 30–80 % of the nigral DA neurons by 8 weeks (Kirik et al. 2002). T-lymphocyte infiltration was related to the degree of neurodegeneration (Sanchez-Guajardo et al. 2010).

In monkeys, neuropathology following WT α-syn overexpression was confined to caudate putamen DA fibers with a limited cell loss in the SN, whereas overexpression of A53T α-syn resulted in robust degeneration of DA cells in SNc within one year (Eslamboli et al. 2007). Overexpression of A53T α-syn produced a long-term increase in microglia, while WT α-syn overexpression increased microglia for more than a year (Barkholt et al. 2012).

Injection of monomeric or oligomeric α-syn into SN induced microgliosis (Wilms et al. 2009; Couch et al. 2011). The use of HA-TAT internalization signal peptide to introduce nitrated α-syn into cells confirmed that microgliosis correlated

with α-syn-induced neurodegeneration (Yu et al. 2010). Distinct strains of α-syn may differentially promote tau inclusions and help explain the heterogeneity of synucleinopathies (Guo et al. 2013).

In Vivo and Behavioral Features Related to Inflammation

Mice overexpressing WT human α-syn under the Thy1 promoter exhibited impairments in sensorimotor function and non-motor symptoms at a young age, including deficits in olfaction, autonomic, digestive, and cognitive function (Fleming et al. 2004). Homozygous mice expressing the A53T mutant human α-syn under the control of the mouse prion promoter developed and displayed severe motor impairments with accumulation of α-syn in brain (Giasson et al. 2002). Homozygous A53T mutant mice exhibited decreased anxiety in 2-month-old animals (George et al. 2008) and increased locomotor activity and altered DA neurotransmission at 7–19-months, while mice expressing human WT or A30P α-syn showed no locomotor changes (Unger et al. 2006). Mice expressing the A53T mutant form of human α-syn exhibited hyperactivity and reduced anxiety-like behavior (Graham and Sidhu 2010). No neuroinflammatory parameters were reported in these transgenic α-syn overexpressor models.

Rats transduced with rAAV-α-syn displayed significant motor impairment when DA neuronal cell loss exceeded 50–60 % (Kirik et al. 2002). When α-syn induced neuronal pathology but not cell death, there was a long-term induction of MHC-II$^+$ microglia. In contrast, when α-syn induced both neuronal pathology and cell death, there was a delayed increase in microglia, which correlated with long-lasting CD68 expression and the reminiscent morphology of peripheral macrophages. A recent study from the same group found that when rats were vaccinated with recombinant α-syn prior receiving the transgenesis of α-syn; this resulted in (a) a high-titer anti-α-syn antibody response on α-syn overexpression, (b) the accumulation of CD4-positive T cells, (c) MHC-II-positive ramified microglia in the SN, (d) long-lasting infiltration of CD4-positive T cells, (e) Foxp3-positive cells throughout the nigrostriatal system, and (f) fewer pathologic aggregates in the striatum versus control animals that had received a mock vaccine. A long-term increase in striatal glial cell-derived neurotrophic factor in striatum and IgG deposition in α-syn-overexpressing cells and neurites in the SN was also observed. These results suggest that a protective vaccination strategy may induce regulatory T cells and activated microglia, and consequently immune tolerance against α-syn (Sanchez-Guajardo et al. 2013b). In rats injected in SN with rAAV-α-syn, functional impairment in the cylinder test and the adjusting steps task was observed after 8 week (Gombash et al. 2013).

rAAV-α-syn-treated monkeys, both WT and mutants, developed motor impairment, including head position bias, compatible with this magnitude of nigrostriatal damage (Kirik et al. 2003). Animals overexpressing the A53T α-syn showed a gradual worsening of motor performance (Eslamboli et al. 2007).

2.3.2 Leucine-rich Repeat Kinase 2 (LRRK2)

LRRK2 is a 2527 amino acid protein that contains functional kinase and GTPase domains, and leucine-rich repeat and WD40 protein-interaction domains (Paisan-Ruiz et al. 2004; Zimprich et al. 2004). It is expressed throughout various brain regions, including SN, basal ganglia, cortex, hippocampus, and cerebellum (Biskup et al. 2006; Healy et al. 2008). Multiple functions for LRRK2 have been suggested, including protein scaffolding, substrate binding, and protein phosphorylation (Drolet et al. 2011) and modulation of chaperone-mediated autophagy (Orenstein et al. 2013), a mechanism that may cause α-syn aggregation.

Mutations in LRRK2 are the most common cause of familial PD and are linked to both autosomal dominant and sporadic forms (Correia Guedes et al. 2010). The most prevalent modification of LRRK2 is the amino acid substitution G2019S in the kinase domain, generating a gain of function. Overexpression of G2019S has been linked to enhanced LRRK2 autophosphorylation and kinase activity (Greggio et al. 2006; Li et al. 2010a, b). A genome-wide study has also identified LRRK2 as a susceptibility locus for Crohn's disease (Danoy et al. 2010).

LRRK2 knockout rats are resistant to DA neurodegeneration from intracranial administration of LPS or adeno-associated virus-mediated transduction of human α-syn, and this resistance correlates with reduced proinflammatory myeloid cells recruited to the brain. These data suggest that knocking down LRRK2 may protect against SN neuronal loss by inhibiting recruitment of proinflammatory myeloid cells (Daher et al. 2014).

Cellular and Cytokine Responses

A role for LRRK2 in immune response was first proposed due to its expression in B-lymphocytes, dendritic cells, and macrophages (Gardet et al. 2010) and isolated mouse microglial cells (Gillardon et al. 2012). LRRK2 levels in microglia were upregulated by LPS, with LRRK2 (1441G) transgenic microglia secreting higher levels of TNF-α, IL-1β, and IL-6 and lower amounts of anti-inflammatory IL-10 than controls. Neurotoxic effects were confirmed when culture medium from LPS-stimulated cells was added to cultured cortical neurons (Gillardon et al. 2012). Knockdown of LRRK2 in microglia reduced LPS-induced TNF-α and iNOS production and decreased the activation of the p38 and NF-κB pathways (Kim et al. 2012). Additional studies confirmed a link between the G2109S LRRK2 mutation and an elevated LPS-induced-inflammatory response that includes TNF-α, IL-1β, IL-6, NF-κβ, and iNOS secretion (Kim et al. 2012; Moehle et al. 2012). Recently, phosphorylation of LRRK2 was shown to involve a TLR-mediated pathway involving Myd88, suggesting a role for LRRK2 in innate immune response (Dzamko et al. 2012).

In Vivo and Behavioral Features Related to Inflammation

Multiple behavioral features have been noted in animal models of LRRK2 mutation. Temporary but not constitutive overexpression of LRRK2 in adult rats impaired DA reuptake by DAT and consequently enhanced locomotor activity (Zhou et al. 2011). Transgenic mice expressing human LRRK2 with an I2020T mutation in the kinase domain exhibited impaired locomotion, reduced striatal DA, fragmented Golgi apparatus in DA neurons, and increased microtubule polymerization, while TH primary neurons derived from the transgenic mouse showed increased frequency of apoptosis and neurites with fewer branches and decreased outgrowth (Maekawa et al. 2012).

Mice lacking LRRK2 exon 41, which encodes the activation hinge of the kinase domain, displayed abnormal behavior (Hinkle et al. 2012). LRRK2 (R1441G) BAC transgenic mice displayed gastrointestinal dysfunction at an early stage (Bichler et al. 2013). These studies did not analyze neuroinflammatory parameters.

Two groups have reported viral LRRK-2-based PD models using adeno- or herpes simplex virus (Lee et al. 2010; Dusonchet et al. 2011). During the first three weeks after injection, LRRK-2 G2019S virus induced a greater loss of TH-positive neurons in the SNc than WT LRRK-2 or EGFP expressing viruses. G2019S LRRK-2 expressing mice, but not WT LRRK2 or EGFP expressing mice, displayed microglia activation in the SNc and striatum (Lee et al. 2010). In the second study, WT or G2019S mutant human LRRK-2 were delivered in adenovirus type 5 vectors and expressed under control of the neuron specific synapsin-1 promoter. Mutant G2019S LRRK-2 caused progressive loss of DA neurons in the SNc with the majority of transduced neurons killed after 6 weeks. Transient inflammation was observed in the striatum at 10 days (Dusonchet et al. 2011). Neuroinflammation and behavior were not analyzed in depth.

2.3.3 Parkin (PARK2), PTEN-induced Putative Kinase 1 (PINK1), and DJ-1

These genes are implicated in mitochondrial function, particularly in stress response pathways. PARK2 acts as a regulator of protein breakdown (Miklya et al. 2014). Mutations in the *parkin* gene, which encodes for an E3 ubiquitin ligase, are the leading cause of early-onset, autosomal recessive parkinsonism (Kitada et al. 1998; Hattori et al. 1998; Lücking et al. 2000). Parkin levels in neurons are associated with protection from cellular stress and cell-cycle regulation (Tran et al. 2011).

PINK1 gene mutations represent the second most common cause of autosomal recessive PD. The gene encodes a 581-amino acid protein with a predicted N-terminal mitochondrial targeting sequence and a conserved serine/threonine kinase domain (Valente et al. 2004). More than 40 PINK1 mutations have been identified in PD patients (Corti et al. 2013). The homozygous form of G309D

PINK1 has been linked to mitochondrial dysfunction and peroxidation damage (Hoepken et al. 2007).

Point mutations (L166P, D149A) in DJ-1 cause rare autosomal recessive PD with early onset. DJ-1 is a redox-sensitive cytosolic chaperone protein that associates with mitochondria and the nucleus upon oxidation. Mutations cause a loss of function of DJ-1 by inducing instability of the dimeric, functional form of the protein, or lack of expression. Mutations also affect the serine protease activity of DJ-1, another crucial function of this protein (Alberio et al. 2012).

Cellular and Cytokine Responses

A role for parkin in glial cells with possible neuroinflammatory consequences has been proposed by in vitro studies. Casarejos et al. (2006) discovered that midbrain neurons cultured from parkin KO mice are more sensitive to rotenone and that the addition of parkin KO microglia to WT neurons increased sensitivity to rotenone. Aged parkin KO mice display fewer astrocytes, more microglia, reduced glial proliferation, and increased pro-apoptotic protein expression (Solano et al. 2008). Parkin levels are regulated by inflammatory signaling: LPS and TNF-α down-regulate parkin expression in macrophages, microglia, and neurons from WT mice blocked by inhibitors of nuclear factor-kappa β, and macrophages isolated from parkin KO mice display increased TNF-α, IL-1α, and iNOS mRNA expressions (Tran et al. 2011).

PINK1 stimulates IL-1β-mediated inflammatory signaling via enhanced TRAF6 and TAK1 (Lee et al. 2012a) and IL-1β-mediated signaling through Tollip and IRAK1 modulation (Lee and Chung 2012).

DJ-1 seems to be an important redox-reactive signaling intermediate controlling oxidative stress associated with ischemia, neuroinflammation, and age-related neurodegeneration (Kahle et al. 2009).

In Vivo and Behavioral Features Related to Inflammation

Parkin. Several groups have generated parkin KO mice (Goldberg et al. 2003; Itier et al. 2003; Von Coelln et al. 2004). Initial characterization failed to note nigral DA neuronal death, although abnormal DA metabolism was noted. Interestingly, catecholaminergic neurons in locus coeruleus of mice with parkin catalytic domain deletion degenerated with an accompanying deficit in the startle response (Von Coelln et al. 2004).

Proteomic studies using parkin-null mice showed marked reduction of mitochondrial respiratory chain proteins and stress response proteins, while several parkin substrates (AIMP2, FBP1, and PARIS) accumulate in the ventral midbrain of parkin-null mice (Ko et al. 2005; Shin et al. 2011). These cellular changes may be responsible for subtle deficits in DA metabolism and behavior. MPTP intoxication in parkin-null mice caused a similar level of DA neuronal toxicity as in

WT mice, although parkin overexpression protects against MPTP (Perez et al. 2005; Paterna et al. 2007; Thomas et al. 2007). Lentiviral-Cre nigral injection into adult parkinflox/flox mice causes acute parkin deletion and progressive nigral neuron death 10 months after the gene deletion. This model also showed pathogenic events caused by accumulation of PARIS including PGC1-α downregulation, and ultimately mitochondrial dysfunction (Shin et al. 2011).

PINK1. PINK1-targeted KOs (Kitada et al. 2007; Gispert et al. 2009) and shRNA-mediated knockdown models (Zhou et al. 2007b) did not replicate robust degenerative phenotypes and mitochondrial defects reported in fly models (Clark et al. 2006). Nevertheless, subtle deficits of nigrostriatal DA transmission and accompanying mild mitochondrial abnormalities were observed. One PINK1 KO model shows that a *Oryzias latipes* fish (known as medaka or Japanese rice fish) model deficient in PINK1 and parkin exhibited late-onset locomotor dysfunction, decreased DA levels, selective degeneration of DA neurons, and defects in mitochondrial activity death (Matsui et al. 2013).

DJ-1. LPS produced similar neuroinflammatory responses in of DJ-1-/- mice and WT mice, and the administration of soluble TNF did not appear to induce neuroinflammatory responses in LPS-treated wild-type or DJ-1-/- mice. Peripheral macrophages from WT and DJ-1-/- mice also displayed similar LPS-induced inflammatory and oxidative stress markers in vitro. The authors concluded that DJ-1 does not play a critical role in protecting DA neurons against inflammation-induced oxidative stress and/or there is compensatory gene expression in the midbrain of DJ-1-/- mice that renders them resistant to the cytotoxic effects triggered by peripheral inflammation (Nguyen et al. 2013).

Mice deficient for both parkin and DJ-1 were crossed with mice deficient for glutathione peroxidase 1, which is reduced in PD brains (Damier et al. 1993). These animals showed higher than normal striatal DA levels in the absence of nigral cell loss than wild-type, glutathione peroxidase 1 (-/-), and Parkin (-/-)DJ-1(-/-) mutant mice. Parkin(-/-)DJ-1(-/-) mice exhibit improved rotarod performance and increased serotonin in the striatum and hippocampus (Hennis et al. 2014).

There has been little analysis of the effects neuroinflammation on specific behavioral features for the genetic models of Parkin, PINK1, or DJ-1.

3 Discussion

3.1 Toxin-based Models

Parkinsonism in patients may develop after exposure to neurotoxins over decades, a feature that cannot be replicated in useable animal models (Nagatsu 1997). Traditional animal models of PD rather rely on toxins that selectively accumulate in SN DA neurons. The extent to which they effectively and reproducibly mimic the entirety of the human condition is controversial. As neurotoxin-induced models of

PD use a single or few injections over a short period and are followed by rapid onset of symptoms, their applicability for studying chronic neuroinflammation is limited (Potashkin et al. 2011) and these models may be best at emulating very late stages of the disease when neurons have died rather than the steps that cause neuronal death.

There is nevertheless clear evidence of neuroinflammation following injection of each commonly used neurotoxin to emulate PD, including MPTP, 6-OHDA, rotenone, and paraquat. While these compounds can cause acute parkinsonism in patients, they each appear to destroy DA neurons in manners different than PD and are likely to trigger stress responses including cytokine release and activation of glial cell types that do not resemble genuine PD. Importantly, each of these toxins are also quite effective at killing cultured DA neurons rapidly in the absence of neuroinflammatory cell types. It may be that the acute toxic effects of these compounds indicate inflammatory responses due to generalized stress responses and do not reveal steps that participate in the loss of neurons in PD.

3.2 Inflammatory Models

Neuroinflammation has been increasingly associated with the development of PD, but a direct cause–effect relationship has not been formally established for bacterial or viral infection-induced development of PD in humans. While inflammatory models, especially LPS, are potent stimulators of microglia and have introduced possible roles for inflammation-mediated DA neurodegeneration, these may not model valid pathogenic steps of the genuine disease. They may, however, provide useful insights into combinatorial models that emulate "multiple hits" that may better model the disorder.

3.3 Genetic Models

Although the majority of PD remains sporadic, specific genetic defects in rare familial cases have provided unique insights into the pathogenesis of PD. Models using PD pathogenic mutations seem more likely to reveal immunological responses involved in pathogenesis. These models, however, have mostly been disappointing in that they do not replicate degenerative features PD, including death of the analogous neuronal populations, and so their use is limited to date.

Our recent work suggests that antigen presentation to T cells might be involved in neuronal death in PD (Cebrián et al. 2014), consistent with studies suggesting that MHC molecules play a role in PD animal models where α-syn is overexpressed by viral transduction (Harms et al. 2013). As treatments that interact with specific T-cell populations already exist for immunological disorders such as multiple sclerosis, perhaps such therapies could be successfully extended to treat PD.

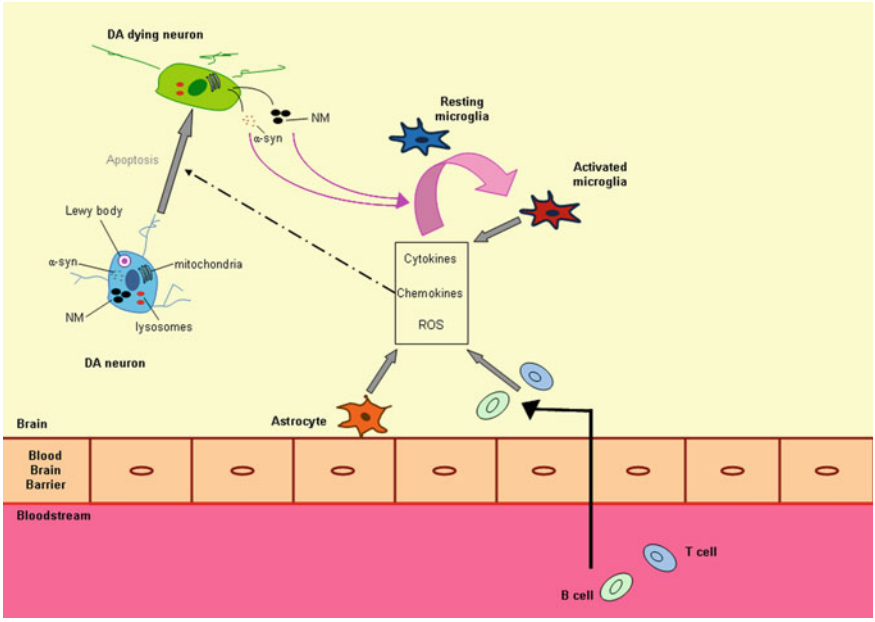

Fig. 1 A neuroinflammatory vicious cycle that may take place in PD. Lymphocytes cross the BBB and penetrate the brain, where they release a variety of proinflammatory factors including cytokines, chemokines, and ROS. Activated glia including astrocytes and microglia also release these substances, which may trigger the cell death of DA neuron through a variety of mechanisms discussed throughout the review. The resulting dying DA neurons produce extracellular α-syn and NM, which in turn further activates astrocytes and microglia, resulting in further tropic signaling for local inflammatory invasion and proliferation, thus providing an ongoing cycle of neurodegeneration.

Alternate approaches including viral-mediated expression of pathogenic mutations, direct injection of disease proteins, or combinatorial genetic and inflammatory substance treatments appear promising and may better characterize the roles of neuroimmune responses.

4 Conclusion

We leave the question posed in our title unanswered: while evidence for nearly a century clearly indicates that neuroinflammation is prominent during PD progression, and many studies show that manipulating inflammatory steps can alter progression in a variety of animal models of PD (Fig. 1; Tufekci et al. 2012; Blandini 2013), it remains unclear which if any steps are required for pathogenesis, or which are stress response mechanisms that provide neuroprotection. Of course, a particular inflammatory cascade could play both roles.

In our opinion, the most fruitful avenue to elucidate these roles is to develop improved PD models that better emulate pathogenesis. Models using PD genetic mutations including viral-mediated expression are to date poor at emulating central features of the PD, such as death of the neurons in the SN, but this technology is still relatively new and improved models will doubtless arrive. In the meantime, combinatorial efforts, such as the combination of an infective agent with mutations, appear promising.

We think that the field needs to keep in focus the genuine complexity of PD. There are now nearly 20 genetic "causes" of PD, and still most patients do not express these mutations, meaning that there is syndrome with multiple etiologies. There are further multiple "hits" that must occur, including aging which features a long list of alterations in protein and organelle turnover; factors that make particular neurons susceptible, likely including pacemaking activity and long axons; a particular targeting of monoaminergic and some cholinergic neurons; and in nearly all cases a synucleinopathy indicating the aggregation of a particular protein, in addition to inflammatory responses. The hopeful news about the requirement of multiple hits in PD pathogenesis is that there may be multiple means to interfere with disease progression.

References

Abeliovich A, Schmitz Y, Fariñas I et al (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25(1):239–252

Akiyama H, McGeer PL (1989) Microglial response to 6-hydroxydopamine-induced substantia nigra lesions. Brain Res 489(2):247–253

Alberio T, Lopiano L and Fasano M (2012) Cellular models to investigate biochemical pathways in Parkinson's disease. FEBS J279. doi:10.1111/j.1742-4658.2012.08516.x

Ambrosi G, Armentero MT, Levandis G et al (2010) Effects of early and delayed treatment with an mGluR5 antagonist on motor impairment, nigrostriatal damage and neuroinflammation in a rodent model of Parkinson's disease. Brain Res Bull 82(1–2):29–38

Armentero MT, Levandis G, Nappi G et al (2006) Peripheral inflammation and neuroprotection: systemic pretreatment with complete Freund's adjuvant reduces 6-hydroxydopamine toxicity in a rodent model of Parkinson's disease. Neurobiol Dis 24(3):492–505

Baldereschi M, Inzitari M, Vanni P et al (2008) Pesticide exposure might be a strong risk factor for Parkinson's disease. Ann Neurol 63(1):128

Barcia C, Ros CM, Annese V et al (2011) IFN-γ signaling, with the synergistic contribution of TNF-α, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. Cell Death Dis 2:e142

Barkholt P, Sanchez-Guajardo V, Kirik D et al (2012) Long-term polarization of microglia upon α-synuclein overexpression in nonhuman primates. Neuroscience 208:85–96

Barnum CJ, Tansey MG (2010) Modeling neuroinflammatory pathogenesis of Parkinson's disease. Prog Brain Res 184:113–132

Betarbet R, Sherer TB, MacKenzie G et al (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3(12):1301–1306

Bichler Z, Lim HC, Zeng L, Tan EK (2013) Non-motor and motor features in LRRK2 transgenic mice. PLoS One 8(7):e70249

Biskup S, Moore DJ, Celsi F et al (2006) Localization of LRRK2 to membranous and vesicular structures in mammalian brain. Ann Neurol 60(5):557–569

Blandini F (2013) Neural and immune mechanisms in the pathogenesis of Parkinson's disease. Funct Neurol 28(1):5

Bobyn J, Mangano EN, Gandhi A et al (2012) Viral-toxin interactions and Parkinson's disease: poly I: C priming enhanced the neurodegenerative effects of paraquat. J Neuroinflammation 9:86

Bové J, Perier C (2012) Neurotoxin-based models of Parkinson's disease. Neuroscience 211:51–76

Braak H, Del Tredici K (2010) Pathophysiology of sporadic Parkinson's disease. Fortschr Neurol Psychiatr 78(Suppl 1):2–4

Brochard V, Combadière B, Prigent A et al (2009) Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 119 (1):182–192

Bronstein DM, Perez-Otano I, Sun V et al (1995) Glia-dependent neurotoxicity and neuroprotection in mesencephalic cultures. Brain Res 704(1):112–116

Bungeroth M, Appenzeller S, Regulin A, Völker W et al (2014) Differential aggregation properties of alpha-synuclein isoforms. Neurobiol Aging S0197–4580(14):00201–00202

Carvey PM, Chang Q, Lipton JW et al (2003) Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease. Front Biosci 8:826–837

Casarejos MJ, Menéndez J, Solano RM et al (2006) Susceptibility to rotenone is increased in neurons from parkin null mice and is reduced by minocycline. J Neurochem 97(4):934–946

Castaño A, Herrera AJ, Cano J et al (1998) Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. J Neurochem 70 (4):1584–1592

Castaño A, Herrera AJ, Cano J et al (2002) The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-alpha, IL-1beta and IFN-gamma. J Neurochem 81(1):150–157

Cebrián C, Zucca FA, Mauri P et al (2014) MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration. Nat Commun 16(5):3633

Chesselet MF (2008) In vivo alpha-synuclein overexpression in rodents: a useful model of Parkinson's disease? Exp Neurol 209(1):22–27

Choi DY, Liu M, Hunter RL et al (2009) Striatal neuroinflammation promotes Parkinsonism in rats. PLoS One 4(5):e5482

Chung YC, Kim SR, Jin BK (2010) Paroxetine prevents loss of nigrostriatal dopaminergic neurons by inhibiting brain inflammation and oxidative stress in an experimental model of Parkinson's disease. J Immunol 185(2):1230–1237

Cicchetti F, Brownell AL, Williams K et al (2002) Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. Eur J Neurosci 15(6):991–998

Cicchetti F, Drouin-Ouellet J, Gross RE (2009) Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? Trends Pharmacol Sci 30 (9):475–483

Cicchetti F, Lapointe N, Roberge-Tremblay A et al (2005) Systemic exposure to paraquat and maneb models early Parkinson's disease in young adult rats. Neurobiol Dis 20(2):360–371

Clark IE, Dodson MW, Jiang C et al (2006) Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 441:1162–1166

Collins LM, Toulouse A, Connor TJ et al (2012) Contributions of central and systemic inflammation to the pathophysiology of Parkinson's disease. Neuropharmacology 62 (7):2154–2168

Conway KA, Harper JD, Lansbury PT Jr (2000a) Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. Biochemistry 39 (10):2552–2563

Conway KA, Lee SJ, Rochet JC et al (2000b) Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 97(2):571–576

Correia Guedes L, Ferreira JJ, Rosa MM et al (2010) Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. Parkinsonism Relat Disord 16 (4):237–242

Corti O, Brice A (2013) Mitochondrial quality control turns out to be the principal suspect in parkin and PINK1-related autosomal recessive Parkinson's disease. Curr Opin Neurobiol 23:100–108

Couch Y, Alvarez-Erviti L, Sibson NR et al (2011) The acute inflammatory response to intranigral α-synuclein differs significantly from intranigral lipopolysaccharide and is exacerbated by peripheral inflammation. J Neuroinflammation 8:166

Cuervo AM, Stefanis L, Fredenburg R et al (2004) Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305(5688):1292–1295

Członkowska A, Kohutnicka M, Kurkowska-Jastrzebska I et al (1996) Microglial reaction in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Parkinson's disease mice model. Neurodegeneration 5(2):137–143

Daher JP, Volpicelli-Daley LA1, Blackburn JP et al (2014) Abrogation of α-synuclein-mediated dopaminergic neurodegeneration in LRRK2-deficient rats. Proc Natl Acad Sci USA (in press)

Damier P, Hirsch EC, Zhang P et al (1993) Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52(1):1–6

Danoy P, Pryce K, Hadler J et al (2010) Association of variants at 1q32 and STAT3 with ankylosing spondylitis suggests genetic overlap with Crohn's disease. PLoS Genet 6(12): e1001195

Dauer W, Kholodilov N, Vila M et al (2002) Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. Proc Natl Acad Sci USA 99(22):14524–14529

Davis GC, Williams AC, Markey SP et al (1979) Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1(3):249–254

Dawson TM, Ko HS, Dawson VL (2010) Genetic animal models of Parkinson's disease. Neuron 66(5):646–661

de Meira Santos Lima M, Braga Reksidler A, Marques Zanata S et al (2006) Different parkinsonism models produce a time-dependent induction of COX-2 in the substantia nigra of rats. Brain Res 1101(1):117–125

De Pablos RM, Herrera AJ, Villarán RF et al (2005) Dopamine-dependent neurotoxicity of lipopolysaccharide in substantia nigra. FASEB J 19(3):407–409

Del Tredici K, Braak H (2012) Lewy pathology and neurodegeneration in premotor Parkinson's disease. Mov Disord 27(5):597–607

Deleidi M, Hallett PJ, Koprich JB et al (2010) The Toll-like receptor-3 agonist polyinosinic: polycytidylic acid triggers nigrostriatal dopaminergic degeneration. J Neurosci 30 (48):16091–16101

Deng H, Yuan L (2014) Genetic variants and animal models in SNCA and Parkinson disease. Ageing Res Rev. 2014 Apr 21. pii: S1568–1637(14):00048–8

Desai VG, Feuers RJ, Hart RW et al (1996) MPP(+)-induced neurotoxicity in mouse is age-dependent: evidenced by the selective inhibition of complexes of electron transport. Brain Res 715(1–2):1–8

Drolet RE, Sanders JM, Kern JT (2011) Leucine-rich repeat kinase 2 (LRRK2) cellular biology: a review of recent advances in identifying physiological substrates and cellular functions. J Neurogenet 25(4):140–151

Dusonchet J, Kochubey O, Stafa K et al (2011) A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. J Neurosci 31(3):907–912

Dutta G, Zhang P, Liu B (2008) The lipopolysaccharide Parkinson's disease animal model: mechanistic studies and drug discovery. Fundam Clin Pharmacol 22(5):453–464

Dzamko N, Inesta-Vaquera F, Zhang J et al (2012) The IkappaB kinase family phosphorylates the Parkinson's disease kinase LRRK2 at Ser935 and Ser910 during Toll-like receptor signaling. PLoS One 7(6):e39132

Emmer KL, Waxman EA, Covy JP et al (2011) E46K human alpha-synuclein transgenic mice develop Lewy-like and tau pathology associated with age-dependent, detrimental motor impairment. J Biol Chem 286(40):35104–35118

Engelhardt B, Coisne C (2011) Fluids and barriers of the CNS establish immune privilege by confining immune surveillance to a two-walled castle moat surrounding the CNS castle. Fluids Barriers CNS 8(1):4

Eslamboli A, Romero-Ramos M, Burger C, Bjorklund T et al (2007) Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 130(Pt 3):799–815

Esposito E, Impellizzeri D, Mazzon E et al (2012) Neuroprotective activities of palmitoylethanolamide in an animal model of Parkinson's disease. PLoS One 7(8):e41880

Fang F, Wirdefeldt K, Jacks A et al (2012) CNS infections, sepsis and risk of Parkinson's disease. Int J Epidemiol 41(4):1042–1049

Fleming SM, Salcedo J, Fernagut PO et al (2004) Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. J Neurosci 24(42):9434–9440

Foix C, Nicolesco J (1925) Anatomie cérébrale. Les noyaux gris centraux et la región Mésencéphalo-sous-optique., Suivi d'un apéndice sur l'anatomie pathologique de la maladie de Parkinson. Masson et Cie, eds (Paris), 508–538

Fortin DL, Nemani VM, Voglmaier SM et al (2005) Neural activity controls the synaptic accumulation of alpha-synuclein. J Neurosci 25(47):10913–10921

Franzén B, Duvefelt K, Jonsson C, Engelhardt B et al (2003) Gene and protein expression profiling of human cerebral endothelial cells activated with tumor necrosis factor-alpha. Brain Res Mol Brain Res 115(2):130–146

Frank-Cannon TC, Alto LT, McAlpine FE et al (2009) Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener 16(4):47

Frank-Cannon TC, Tran T, Ruhn KA et al (2008) Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. J Neurosci 28(43):10825–10834

Fulceri F, Biagioni F, Lenzi P et al (2006) Nigrostriatal damage with 6-OHDA: validation of routinely applied procedures. Ann N Y Acad Sci 1074:344–348

Gao JJ, Diesl V, Wittmann T et al (2002a) Regulation of gene expression in mouse macrophages stimulated with bacterial CpG-DNA and lipopolysaccharide. J Leukoc Biol 72(6):1234–1245

Gao HM, Hong JS, Zhang W et al (2002b) Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. J Neurosci 22(3):782–790

Gao HM, Kotzbauer PT, Uryu K et al (2008) Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. J Neurosci 28(30):7687–7698

Gao HM, Liu B, Hong JS (2003) Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J Neurosci 23(15):6181–6187

Gao HM, Zhang F, Zhou H et al (2011) Neuroinflammation and α-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. Environ Health Perspect 119(6):807–814

Gardet A, Benita Y, Li C et al (2010) LRRK2 is involved in the IFN-gamma response and host response to pathogens. J Immunol 185(9):5577–5585

Gayle DA, Ling Z, Tong C et al (2002) Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. Brain Res Dev Brain Res 133(1):27–35

Geng Y, Fang M, Wang J et al (2011) Triptolide down-regulates COX-2 expression and PGE2 release by suppressing the activity of NF-κB and MAP kinases in lipopolysaccharide-treated PC12 cells. Phytother Res 26(3):337–343

George S, van den Buuse M, San Mok S, Masters CL et al (2008) Alpha-synuclein transgenic mice exhibit reduced anxiety-like behaviour. Exp Neurol 210(2):788–792

Ghosh A, Kanthasamy A, Joseph J et al (2012) Anti-inflammatory and neuroprotective effects of an orally active apocynin derivative in pre-clinical models of Parkinson's disease. J Neuroinflammation 9:241

Giasson BI, Duda JE, Quinn SM et al (2002) Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. Neuron 34(4):521–533

Gillardon F, Schmid R, Draheim H (2012) Parkinson's disease-linked leucine-rich repeat kinase 2 (R1441G) mutation increases proinflammatory cytokine release from activated primary microglial cells and resultant neurotoxicity. Neuroscience 208:41–48

Gispert S, Ricciardi F, Kurz A et al (2009) Parkinson phenotype in aged PINK1-deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. PloS ONE 4:e5777

Glinka Y, Gassen M, Youdim MB (1997) Mechanism of 6-hydroxydopamine neurotoxicity. J Neural Transm Suppl 50:55–66

Goes AT, Souza LC, Filho CB et al (2014) Neuroprotective effects of swimming training in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. Neuroscience 256C:61–71

Goldberg MS, Fleming SM, Palacino JJ et al (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278:43628–43635

Gombash SE, Manfredsson FP, Kemp CJ et al (2013) Morphological and behavioral impact of AAV2/5-mediated overexpression of human wildtype alpha-synuclein in the rat nigrostriatal system. PLoS One 8(11):e81426

Gomez-Isla T, Irizarry MC, Mariash A, Cheung B et al (2003) Motor dysfunction and gliosis with preserved dopaminergic markers in human alpha-synuclein A30P transgenic mice. Neurobiol Aging 24(2):245–258

Gomide V, Bibancos T, Chadi G (2005) Dopamine cell morphology and glial cell hypertrophy and process branching in the nigrostriatal system after striatal 6-OHDA analyzed by specific sterological tools. Int J Neurosci 115(4):557–582

González H, Contreras F, Prado C, Elgueta D et al (2013) Dopamine receptor D3 expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's disease. J Immunol 190(10):5048–5056

Graham DR, Sidhu A (2010) Mice expressing the A53T mutant form of human alpha-synuclein exhibit hyperactivity and reduced anxiety-like behavior. J Neurosci Res 88 (8):1777–1783

Greggio E, Jain S, Kingsbury A et al (2006) Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. Neurobiol Dis 23(2):329–341

Grünblatt E, Mandel S, Maor G et al (2001) Gene expression analysis in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice model of Parkinson's disease using cDNA microarray: effect of R-apomorphine. J Neurochem 78(1):1–12

Guo JL, Covell DJ, Daniels JP et al (2013) Distinct α-synuclein strains differentially promote tau inclusions in neurons. Cell 154(1):103–117

Gupta A, Kumar A, Kulkarni SK (2011) Targeting oxidative stress, mitochondrial dysfunction and neuroinflammatory signaling by selective cyclooxygenase (COX)-2 inhibitors mitigates MPTP-induced neurotoxicity in mice. Prog Neuropsychopharmacol Biol Psychiatry 35 (4):974–981

Gupta SP, Patel S, Yadav S et al (2010) Involvement of nitric oxide in maneb- and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? Neurochem Res 35(8):1206–1213

Hancock DB, Martin ER, Mayhew GM et al (2008) Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. BMC Neurol 8:6. doi:10.1186/1471-2377-8-6

Harkavyi A, Abuirmeileh A, Lever R et al (2008) Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. J Neuroinflammation 5:19

Harms AS, Barnum CJ, Ruhn KA et al (2011) Delayed dominant-negative TNF gene therapy halts progressive loss of nigral dopaminergic neurons in a rat model of Parkinson's disease. Mol Ther 19(1):46–52

Harms AS, Cao S, Rowse AL et al (2013) MHCII is required for α-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. J Neurosci 33 (23):9592–9600

Hattori N, Kitada T, Matsumine H et al (1998) Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the Parkin gene in affected individuals. Ann Neurol 44(6):935–941

He Y, Appel S, Le W (2001) Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. Brain Res 909(1–2):187–193

Healy DG, Falchi M, O'Sullivan SS et al (2008) Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 7 (7):583–590

Hennis MR, Marvin MA, Taylor CM, Goldberg MS (2014) Surprising behavioral and neurochemical enhancements in mice with combined mutations linked to Parkinson's disease. Neurobiol Dis 62:113–123

Henry J, Smeyne RJ, Jang H et al (2010) Parkinsonism and neurological manifestations of influenza throughout the 20th and 21st centuries. Parkinsonism Relat Disord 16(9):566–571

Hernández-Romero MC, Argüelles S, Villarán RF et al (2008) Simvastatin prevents the inflammatory process and the dopaminergic degeneration induced by the intranigral injection of lipopolysaccharide. J Neurochem 105(2):445–459

Herva ME, Zibaee S, Fraser G, Barker RA et al (2014) Anti-amyloid compounds inhibit alpha-synuclein aggregation induced by Protein Misfolding Cyclic Amplification (PMCA). J Biol Chem (in press)

Hinkle KM, Yue M, Behrouz B et al (2012) LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. Mol Neurodegener 7:25

Hirsch EC, Hunot S (2009) Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol 8(4):382–397

Hoban DB, Connaughton E, Connaughton C et al (2013) Further characterisation of the LPS model of Parkinson's disease: a comparison of intra-nigral and intra-striatal lipopolysaccharide administration on motor function, microgliosis and nigrostriatal neurodegeneration in the rat. Brain Behav Immun 27(1):91–100

Hoepken HH, Gispert S, Morales B et al (2007) Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. Neurobiol Dis 25:401–411

Hunter RL, Cheng B, Choi DY et al (2009) Intrastriatal lipopolysaccharide injection induces parkinsonism in C57/B6 mice. J Neurosci Res 87(8):1913–1921

Iravani MM, Leung CC, Sadeghian M et al (2005) The acute and the long-term effects of nigral lipopolysaccharide administration on dopaminergic dysfunction and glial cell activation. Eur J Neurosci 22(2):317–330

Itier JM, Ibanez P, Mena MA et al (2003) Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet 12:2277–2291

Izumi Y, Ezumi M, Takada-Takatori Y, Akaike A et al (2014) Endogenous dopamine is involved in the herbicide paraquat-induced dopaminergic cell death. Toxicol Sci (in press)

Janezic S, Threlfell S, Dodson PD, Dowie MJ et al (2013) Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. Proc Natl Acad Sci USA 110 (42):E4016–E4025

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79(4):368–376

Johnston LC, Su X, Maguire-Zeiss K et al (2008) Human interleukin-10 gene transfer is protective in a rat model of Parkinson's disease. Mol Ther 16(8):1392–1399

Jung BD, Shin EJ, Nguyen XK et al (2010) Potentiation of methamphetamine neurotoxicity by intrastriatal lipopolysaccharide administration. Neurochem Int 56(2):229–244

Kahle PJ, Waak J, Gasser T (2009) DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. Free Radic Biol Med 47(10):1354–1361

Kamel F, Tanner C, Umbach D et al (2007) Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am J Epidemiol 165(4):364–374

Kasten M, Klein C (2013) The many faces of alpha-synuclein mutations. Mov Disord 28 (6):697–701

Kim B, Yang MS, Choi D et al (2012) Impaired inflammatory responses in murine Lrrk2-knockdown brain microglia. PLoS One 7(4):e34693

Kirik D, Annett LE, Burger C et al (2003) Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease. Proc Natl Acad Sci USA 100(5):2884–2889

Kirik D, Rosenblad C, Burger C et al (2002) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J Neurosci 22(7):2780–2791

Kitada T, Asakawa S, Hattori N et al (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392(6676):605–608

Kitada T, Pisani A, Porter DR et al (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc Natl Acad Sci 104:11441–11446

Klementiev B, Li S, Korshunova I et al (2014) Anti-inflammatory properties of a novel peptide interleukin 1 receptor antagonist. J Neuroinflammation 11:27

Klintworth H, Garden G, Xia Z (2009) Rotenone and paraquat do not directly activate microglia or induce inflammatory cytokine release. Neurosci Lett 462(1):1–5

Ko HS, von Coelln R, Sriram SR et al (2005) Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, pp38/JTV-1, leads to catecholaminergic cell death. J Neurosci 25:7968–7978

Kopin IJ (1987) MPTP: an industrial chemical and contaminant of illicit narcotics stimulates a new era in research on Parkinson's disease. Environ Health Perspect 75:45–51

Koprich JB, Reske-Nielsen C, Mithal P et al (2008) Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J Neuroinflammation 5:8

Kortekaas R, Leenders KL, van Oostrom JC et al (2005) Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann Neurol 57(2):176–179

Krüger R, Kuhn W, Müller T et al (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Ann Neurol 18(2):106–108

Kunikowska G, Jenner P (2001) 6-Hydroxydopamine-lesioning of the nigrostriatal pathway in rats alters basal ganglia mRNA for copper, zinc- and manganese-superoxide dismutase, but not glutathione peroxidase. Brain Res 922(1):51–64

Kurkowska-Jastrzebska I, Wrońska A, Kohutnicka M et al (1999) The inflammatory reaction following 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine intoxication in mouse. Exp Neurol 156(1):50–61

L'Episcopo F, Tirolo C, Caniglia S et al (2010) Combining nitric oxide release with anti-inflammatory activity preserves nigrostriatal dopaminergic innervation and prevents motor impairment in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. J Neuroinflammation. 7:83

L'Episcopo F, Tirolo C, Testa N et al (2011) Switching the microglial harmful phenotype promotes lifelong restoration of subtantia nigra dopaminergic neurons from inflammatory neurodegeneration in aged mice. Rejuvenation Res 14(4):411–424

Langston JW, Forno LS, Tetrud J et al (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 46(4):598–605

Larsen KE, Schmitz Y, Troyer MD et al (2006) Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. J Neurosci 26(46):11915–11922

Lee HJ, Chung KC (2012) PINK1 positively regulates IL-1β-mediated signaling through Tollip and IRAK1 modulation. J Neuroinflammation 9:271

Lee HJ, Jang SH, Kim H et al (2012a) PINK1 stimulates interleukin-1β-mediated inflammatory signaling via the positive regulation of TRAF6 and TAK1. Cell Mol Life Sci 69 (19):3301–3315

Lee BD, Shin JH, VanKampen J et al (2010) Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. Nat Med 16(9):998–1000

Lee MK, Stirling W, Xu Y et al (2002) Human alpha-synuclein-harboring familial Parkinson's disease-linked Ala-53 –> Thr mutation causes neurodegenerative disease with alpha-synuclein aggregation in transgenic mice. Proc Natl Acad Sci U S A. 99(13):8968–8973

Lee JK, Tran T, Tansey MG (2009) Neuroinflammation in Parkinson's disease. J Neuroimmune Pharmacol 4(4):419–429

Lee KW, Zhao X, Im JY et al (2012b) Apoptosis signal-regulating kinase 1 mediates MPTP toxicity and regulates glial activation. PLoS One 7(1):e29935

Li Z, Arnaud L, Rockwell P et al (2004) A single amino acid substitution in a proteasome subunit triggers aggregation of ubiquitinated proteins in stressed neuronal cells. J Neurochem 90 (1):19–28

Li M, Dai FR, Du XP et al (2012) Neuroprotection by silencing Inos expression in a 6-OHDA model of Parkinson's disease. J Mol Neurosci 48(1):225–233

Li X, Moore DJ, Xiong Y et al (2010a) Reevaluation of phosphorylation sites in the Parkinson disease-associated leucine-rich repeat kinase 2. J Biol Chem 285(38):29569–29576

Li X, Patel JC, Wang J et al (2010b) Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. J Neurosci 30(5):1788–1797

Lin X, Parisiadou L, Sgobio C et al (2012) Conditional expression of Parkinson's disease-related mutant α-synuclein in the midbrain dopaminergic neurons causes progressive neurodegeneration and degradation of transcription factor nuclear receptor related 1. J Neurosci 32 (27):9248–9264

Ling ZD, Chang Q, Lipton JW et al (2004) Combined toxicity of prenatal bacterial endotoxin exposure and postnatal 6-hydroxydopamine in the adult rat midbrain. Neuroscience 124 (3):619–628

Liou HH, Chen RC, Tsai YF (1996) Effects of paraquat on the substantia nigra of the Wistar rats: neurochemical, histological, and behavioral studies. Toxicol Appl Pharmacol 137(1):34–41

Liu M, Bing G (2011) Lipopolysaccharide animal models for Parkinson's disease. Parkinsons Dis 2011:327089

Liu J, Guo YM, Hirokawa M et al (2012) A synthetic double-stranded RNA, poly I:C, induces a rapid apoptosis of human CD34(+) cells. Exp Hematol 40(4):330–341

Liu J, Wang MW, Gu P et al (2010) Microglial activation and age-related dopaminergic neurodegeneration in MPTP-treated SAMP8 mice. Brain Res 1345:213–220

Lücking CB, Dürr A, Bonifati V et al (2000) Association between early-onset Parkinson's disease and mutations in the parkin gene. N Engl J Med 342(21):1560–1567

Luk KC, Kehm V, Carroll J et al (2012a) Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338(6109):949–953

Luk KC, Kehm VM, Zhang B et al (2012b) Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. J Exp Med 209(5):975–986

Machado A, Herrera AJ, Venero JL et al (2011) Inflammatory animal model for Parkinson's disease: the intranigral injection of LPS induced the inflammatory process along with the selective degeneration of nigrostriatal dopaminergic neurons. ISRN Neurol 2011:476158

Maekawa T, Mori S, Sasaki Y et al (2012) The I2020T Leucine-rich repeat kinase 2 transgenic mouse exhibits impaired locomotive ability accompanied by dopaminergic neuron abnormalities. Mol Neurodegener 7:15

Mangano EN, Litteljohn D, So R et al (2012) Interferon-γ plays a role in paraquat-induced neurodegeneration involving oxidative and proinflammatory pathways. Neurobiol Aging 33 (7):1411–1426

Manning-Bog AB, McCormack AL, Li J et al (2002) The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. J Biol Chem 277 (3):1641–1644

Marinova-Mutafchieva L, Sadeghian M, Broom L et al (2009) Relationship between microglial activation and dopaminergic neuronal loss in the substantia nigra: a time course study in a 6-hydroxydopamine model of Parkinson's disease. J Neurochem 110(3):966–975

Martin LJ, Pan Y, Price AC et al (2006) Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. J Neurosci 26(1):41–50

Masliah E, Rockenstein E, Veinbergs I et al (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287 (5456):1265–1269

Masuda-Suzukake M, Nonaka T, Hosokawa M, Oikawa T et al (2013) Prion-like spreading of pathological α-synuclein in brain. Brain 136(Pt 4):1128–1138

Matsui H, Gavinio R, Asano T et al (2013) PINK1 and Parkin complementarily protect dopaminergic neurons in vertebrates. Hum Mol Genet 22(12):2423–2434

Mazzio EA, Reams RR, Soliman KF (2004) The role of oxidative stress, impaired glycolysis and mitochondrial respiratory redox failure in the cytotoxic effects of 6-hydroxydopamine in vitro. Brain Res 1–2:24–29

McClain JA, Phillips LL, Fillmore HL et al (2009) Increased MMP-3 and CTGF expression during lipopolysaccharide-induced dopaminergic neurodegeneration. Neurosci Lett 460(1):27–31

McCoy MK, Martinez TN, Ruhn KA et al (2006) Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. J Neurosci 26(37):9365–9375

McCoy MK, Tansey MG (2008) TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflammation 5:45

McGeer PL, Itagaki S, Boyes BE et al (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38(8):1285–1291

McGeer PL, McGeer EG (1998) Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. Alzheimer Dis Assoc Disord 12(Suppl 2):S1–S6

McGeer PL, Schwab C, Parent A et al (2003) Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. Ann Neurol 54 (5):599–604

Mena MA, García de Yébenes J (2008) Glial cells as players in parkinsonism: the "good," the "bad," and the "mysterious" glia. Neuroscientist 14(6):544–560

Miake H, Mizusawa H, Iwatsubo T, Hasegawa M (2002) Biochemical characterization of the core structure of alpha-synuclein filaments. J Biol Chem 277(21):19213–19219

Miklya I, Goltl P, Hafenscher F et al (2014) The role of parkin in Parkinson's disease. Neuropsychopharmacol Hung 16(2):67–76

Miller RM, Kiser GL, Kaysser-Kranich T et al (2007) Wild-type and mutant alpha-synuclein induce a multi-component gene expression profile consistent with shared pathophysiology in different transgenic mouse models of PD. Exp Neurol 204(1):421–432

Mitra S, Chakrabarti N, Bhattacharyya A (2011) Differential regional expression patterns of α-synuclein, TNF-α, and IL-1β; and variable status of dopaminergic neurotoxicity in mouse brain after Paraquat treatment. J Neuroinflammation 8:163

Moehle MS, Webber PJ, Tse T et al (2012) LRRK2 inhibition attenuates microglial inflammatory responses. J Neurosci 32(5):1602–1611

Mogi M, Togari A, Tanaka K et al (2000) Increase in level of tumor necrosis factor-alpha in 6-hydroxydopamine-lesioned striatum in rats is suppressed by immunosuppressant FK506. Neurosci Lett 289(3):165–168

Mosharov EV, Staal RG, Bové J et al (2006) Alpha-synuclein overexpression increases cytosolic catecholamine concentration. J Neurosci 26(36):9304–9311

Mount MP, Lira A, Grimes D et al (2007) Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. J Neurosci 27(12):3328–3337

Mulcahy P, O'Doherty A, Paucard A, O'Brien T et al (2013) The behavioural and neuropathological impact of intranigral AAV-α-synuclein is exacerbated by systemic infusion of the Parkinson's disease-associated pesticide, rotenone, in rats. Behav Brain Res 15 (243):6–15

Na SJ, DiLella AG, Lis EV et al (2010) Molecular profiling of a 6-hydroxydopamine model of Parkinson's disease. Neurochem Res 35(5):761–772

Nagatsu T (1997) Isoquinoline neurotoxins in the brain and Parkinson's disease. Neurosci Res 29 (2):99–111

Nemani VM, Lu W, Berge V et al (2010) Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. Neuron 65(1):66–79

Nguyen TA, Frank-Cannon T, Martinez TN et al (2013) Analysis of inflammation-related nigral degeneration and locomotor function in DJ-1(-/-) mice. J Neuroinflammation 10:50

Oestreicher E, Sengstock GJ, Riederer P et al (1994) Degeneration of nigrostriatal dopaminergic neurons increases iron within the substantia nigra: a histochemical and neurochemical study. Brain Res 660(1):8–18

Ohnuki T, Nakamura A, Okuyama S et al (2010) Gene expression profiling in progressively MPTP-lesioned macaques reveals molecular pathways associated with sporadic Parkinson's disease. Brain Res 1346:26–42

Olson JK, Miller SD (2004) Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173(6):3916–3924

Orenstein SJ, Kuo SH, Tasset I et al (2013) Interplay of LRRK2 with chaperone-mediated autophagy. Nat Neurosci 16(4):394–406

Pabon MM, Bachstetter AD, Hudson CE et al (2011) CX3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. J Neuroinflammation 8:9

Paisán-Ruíz C, Jain S, Evans EW et al (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44(4):595–600

Park B, Yang J, Yun N et al (2010) Proteomic analysis of expression and protein interactions in a 6-hydroxydopamine-induced rat brain lesion model. Neurochem Int 57(1):16–32

Paterna JC, Leng A, Weber E et al (2007) DJ-1 and Parkin modulate dopamine-dependent behavior and inhibit MPTP-induced nigral dopamine neuron loss in mice. Mol Ther 15:698–704

Perez FA, Curtis WR, Palmiter RD (2005) Parkin-deficient mice are not more sensitive to 6-hydroxydopamine or methamphetamine neurotoxicity. BMC Neurosci 6:71

Perumal AS, Gopal VB, Tordzro WK et al (1992) Vitamin E attenuates the toxic effects of 6-hydroxydopamine on free radical scavenging systems in rat brain. Brain Res Bull 29 (5):699–701

Pierre SR, Lemmens MA, Figueiredo-Pereira ME (2009) Subchronic infusion of the product of inflammation prostaglandin J2 models sporadic Parkinson's disease in mice. J Neuroinflammation 6:18

Polymeropoulos MH, Lavedan C, Leroy E et al (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276(5321):2045–2047

Poon HF, Frasier M, Shreve N et al (2005) Mitochondrial associated metabolic proteins are selectively oxidized in A30P alpha-synuclein transgenic mice–a model of familial Parkinson's disease. Neurobiol Dis 18(3):492–498

Potashkin JA, Blume SR, Runkle NK (2011) Limitations of animal models of Parkinson's disease. Parkinsons Dis 2011:658083

Pott Godoy MC, Tarelli R, Ferrari CC et al (2008) Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. Brain 131(Pt 7):1880–1894

Purisai MG, McCormack AL, Cumine S et al (2007) Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration. Neurobiol Dis 25(2):392–400

Pycock CJ (1980) Turning behaviour in animals. Neuroscience 5(3):461–514

Qin L, Liu Y, Wang T et al (2004) NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem 279 (2):1415–1421
Qin L, Wu X, Block ML et al (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55(5):453–462
Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145
Ransom BR, Kunis DM, Irwin I et al (1987) Astrocytes convert the parkinsonism inducing neurotoxin, MPTP, to its active metabolite, MPP+. Neurosci Lett 75(3):323–328
Reynolds AD, Stone DK, Hutter JA, Benner EJ et al (2010) Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. J Immunol 184(5):2261–2271
Rezai-Zadeh K, Gate D, Town T (2009) CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease? J Neuroimmune Pharmacol 4(4):462–475
Riachi NJ, LaManna JC, Harik SI (1989) Entry of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine into the rat brain. J Pharmacol Exp Ther 249(3):744–748
Rochet JC, Outeiro TF, Conway KA et al (2004) Interactions among alpha-synuclein, dopamine, and biomembranes: some clues for understanding neurodegeneration in Parkinson's disease. J Mol Neurosci 23(1–2):23–34
Rockenstein E, Mallory M, Hashimoto M et al (2002) Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. J Neurosci Res 68(5):568–578
Rockenstein E, Nuber S, Overk CR, Ubhi K et al (2014) Accumulation of oligomer-prone α-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain (in press)
Ross OA, Braithwaite AT, Skipper LM et al (2008) Genomic investigation of alpha-synuclein multiplication and parkinsonism. Ann Neurol 63(6):743–750
Roy A, Ghosh A, Jana A et al (2012) Sodium phenylbutyrate controls neuroinflammatory and antioxidant activities and protects dopaminergic neurons in mouse models of Parkinson's disease. PLoS One 7(6):e38113
Sadeghian M, Marinova-Mutafchieva L, Broom L et al (2012) Full and partial peroxisome proliferation-activated receptor-γ agonists, but not δ agonist, rescue of dopaminergic neurons in the 6-OHDA parkinsonian model is associated with inhibition of microglial activation and MMP expression. J Neuroimmunol 246(1–2):69–77
Salama M, Arias-Carrión O (2011) Natural toxins implicated in the development of Parkinson's disease. Ther Adv Neurol Disord 4(6):361–373
Sanchez-Guajardo V, Annibali A, Jensen PH et al (2013a) α-Synuclein vaccination prevents the accumulation of parkinson disease-like pathologic inclusions in striatum in association with regulatory t cell recruitment in a rat model. J Neuropathol Exp Neurol 72(7):624–645
Sanchez-Guajardo V, Barnum CJ, Tansey MG et al (2013b) Neuroimmunological processes in Parkinson's disease and their relation to α-synuclein: microglia as the referee between neuronal processes and peripheral immunity. ASN Neuro 5(2):113–139
Sanchez-Guajardo V, Febbraro F, Kirik D et al (2010) Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. PLoS One 5(1):e8784
Sánchez-Pernaute R, Ferree A, Cooper O et al (2004) Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. J Neuroinflammation 1(1):6
Schober A (2004) Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. Cell Tissue Res 318(1):215–224
Sekiyama K, Sugama S, Fujita M et al (2012) Neuroinflammation in Parkinson's disease and related disorders: a lesson from genetically manipulated mouse models of α-synucleinopathies. Parkinsons Dis. doi:10.1155/2012/271732

Selvakumar GP, Janakiraman U, Essa MM, Thenmozhi AJ et al (2014) Escin attenuates behavioral impairments, oxidative stress and inflammation in a chronic MPTP/probenecid mouse model of Parkinson′s disease. Brain Res S0006–8993(14):00358

Shaikh SB, Nicholson LF (2009) Effects of chronic low dose rotenone treatment on human microglial cells. Mol Neurodegener 4:55

Sherer TB, Betarbet R, Kim JH et al (2003a) Selective microglial activation in the rat rotenone model of Parkinson's disease. Neurosci Lett 341(2):87–90

Sherer TB, Betarbet R, Testa CM et al (2003b) Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 23(34):10756–10764

Sherer TB, Kim JH, Betarbet R et al (2003c) Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. Exp Neurol 179 (1):9–16

Shimizu K, Ohtaki K, Matsubara K et al (2001) Carrier-mediated processes in blood–brain barrier penetration and neural uptake of paraquat. Brain Res 906(1–2):135–142

Shimoji M, Pagan F, Healton EB et al (2009) CXCR4 and CXCL12 expression is increased in the nigro-striatal system of Parkinson's disease. Neurotox Res 16(3):318–328

Shin JH, Ko HS, Kang H et al (2011) PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. Cell 144:689–702

Sindhu KM, Saravanan KS, Mohanakumar KP (2005) Behavioral differences in a rotenone-induced hemiparkinsonian rat model developed following intranigral or median forebrain bundle infusion. Brain Res 1–2:25–34

Singleton AB, Farrer M, Johnson J et al (2003) Alpha-Synuclein locus triplication causes Parkinson's disease. Science 302(5646):841

Solano RM, Casarejos MJ, Menéndez-Cuervo J et al (2008) Glial dysfunction in parkin null mice: effects of aging. J Neurosci 28(3):598–611

Spillantini MG, Schmidt ML, Lee VM et al (1997) Alpha-synuclein in Lewy bodies. Nature 388 (6645):839–840

Sriram K, Matheson JM, Benkovic SA et al (2002) Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. FASEB J 16 (11):1474–1476

Staal RG, Hananiya A, Sulzer D (2008) PKC theta activity maintains normal quantal size in chromaffin cells. J Neurochem 105(5):1635–1641

Su X, Maguire-Zeiss KA, Giuliano R et al (2008) Synuclein activates microglia in a model of Parkinson's disease. Neurobiol Aging 29(11):1690–1701

Sulzer D, Surmeier DJ (2013) Neuronal vulnerability, pathogenesis, and Parkinson's disease. Mov Disord 28(1):41–50

Swanson CR, Joers V, Bondarenko V et al (2011) The PPAR-γ agonist pioglitazone modulates inflammation and induces neuroprotection in parkinsonian monkeys. J Neuroinflammation 8:91

Tanaka S, Ishii A, Ohtaki H et al (2013) Activation of microglia induces symptoms of Parkinson's disease in wild-type, but not in IL-1 knockout mice. J Neuroinflammation 10(1):143

Tansey MG, Goldberg MS (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37(3):510–518

Taylor JM, Main BS, Crack PJ (2013) Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease. Neurochem Int 62(5):803–819

Theodore S, Cao S, McLean PJ et al (2008) Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J Neuropathol Exp Neurol 67(12):1149–1158

Thomas B, von Coelln R, Mandir AS et al (2007) MPTP and DSP-4 susceptibility of substantia nigra and locus coeruleus catecholaminergic neurons in mice is independent of parkin activity. Neurobiol Dis 26:312–322

Thornton E, Vink R (2012) Treatment with a substance P receptor antagonist is neuroprotective in the intrastriatal 6-hydroxydopamine model of early Parkinson's disease. PLoS One 7(4): e34138

Tobón-Velasco JC, Limón-Pacheco JH, Orozco-Ibarra M, Macías-Silva M et al (2013) 6-OHDA-induced apoptosis and mitochondrial dysfunction are mediated by early modulation of intracellular signals and interaction of Nrf2 and NF-κB factors. Toxicology 8(304):109–119
Tofaris GK, Garcia Reitböck P, Humby T et al (2006) Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J Neurosci v26(15):3942–50
Tomás-Camardiel M, Venero JL, de Pablos RM et al (2004) In vivo expression of aquaporin-4 by reactive microglia. J Neurochem 91(4):891–899
Tran TA, Nguyen AD, Chang J et al (2011) Lipopolysaccharide and tumor necrosis factor regulate Parkin expression via nuclear factor-kappa B. PLoS One 6(8):e23660
Tufekci KU, Genc S, Genc K (2011) The endotoxin-induced neuroinflammation model of Parkinson's disease. Parkinsons Dis 2011:487450
Tufekci KU, Meuwissen R, Genc S et al (2012) Inflammation in Parkinson's disease. Adv Protein Chem Struct Biol. doi:10.1016/B978-0-12-398314-5.00004-0
Ulusoy A, Decressac M, Kirik D et al (2010) Viral vector-mediated overexpression of α-synuclein as a progressive model of Parkinson's disease. Prog Brain Res 184:89–111
Unger EL, Eve DJ, Perez XA et al (2006) Locomotor hyperactivity and alterations in dopamine neurotransmission are associated with overexpression of A53T mutant human alpha-synuclein in mice. Neurobiol Dis. 21(2):431–443
Valente EM, Abou-Sleiman PM, Caputo V et al (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304:1158–1160
Vázquez-Claverie M, Garrido-Gil P, San Sebastián W et al (2009) Acute and chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administrations elicit similar microglial activation in the substantia nigra of monkeys. J Neuropathol Exp Neurol 68(9):977–984
Volpicelli-Daley LA, Luk KC, Patel TP et al (2011) Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron 72(1):57–71
von Coelln R, Kügler S, Bähr M, Weller M et al (2001) Rescue from death but not from functional impairment: caspase inhibition protects dopaminergic cells against 6-hydroxydopamine-induced apoptosis but not against the loss of their terminals. J Neurochem 77(1):263–273
Von Coelln R, Thomas B, Savitt JM et al (2004) Loss of locus coeruleus neurons and reduced startle in parkin null mice. Proc Natl Acad Sci 101:10744–10749
Wachter B, Schürger S, Rolinger J et al (2010) Effect of 6-hydroxydopamine (6-OHDA) on proliferation of glial cells in the rat cortex and striatum: evidence for de-differentiation of resident astrocytes. Cell Tissue Res. 342(2):147–160
Wang AL, Liou YM, Pawlak CR et al (2010) Involvement of NMDA receptors in both MPTP-induced neuroinflammation and deficits in episodic-like memory in Wistar rats. Behav Brain Res 208(1):38–46
Wang Q, Shin EJ, Nguyen XK et al (2012) Endogenous dynorphin protects against neurotoxin-elicited nigrostriatal dopaminergic neuron damage and motor deficits in mice. J Neuroinflammation 9:124
Wang XJ, Zhang S, Yan ZQ et al (2011) Impaired CD200-CD200R-mediated microglia silencing enhances midbrain dopaminergic neurodegeneration: roles of aging, superoxide, NADPH oxidase, and p38 MAPK. Free Radic Biol Med 50(9):1094–1106
Watson MB, Richter F, Lee SK et al (2012) Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. Exp Neurol 237(2):318–334
Wilms H, Rosenstiel P, Romero-Ramos M et al (2009) Suppression of MAP kinases inhibits microglial activation and attenuates neuronal cell death induced by alpha-synuclein protofibrils. Int J Immunopathol Pharmacol 22(4):897–909
Wu SY, Wang TF, Yu L et al (2011) Running exercise protects the substantia nigra dopaminergic neurons against inflammation-induced degeneration via the activation of BDNF signaling pathway. Brain Behav Immun 25(1):135–146
Yadav S, Gupta SP, Srivastava G et al (2012) Role of secondary mediators in caffeine-mediated neuroprotection in maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. Neurochem Res 37(4):875–884

Yasuda Y, Shimoda T, Uno K et al (2008) The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. J Neuroimmunol 204 (1–2):43–51

Yu Z, Xu X, Xiang Z et al (2010) Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. PLoS One 5(4):e9956

Zarranz JJ, Alegre J, Gómez-Esteban JC et al (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55(2):164–173

Zhang ZJ, Cheang LC, Wang MW et al (2011a) Ethanolic extract of fructus Alpinia oxyphylla protects against 6-hydroxydopamine-induced damage of PC12 cells in vitro and dopaminergic neurons in zebrafish. Cell Mol Neurobiol 32(1):27–40

Zhang S, Wang XJ, Tian LP et al (2011b) CD200-CD200R dysfunction exacerbates microglial activation and dopaminergic neurodegeneration in a rat model of Parkinson's disease. J Neuroinflammation 8:154

Zhang W, Phillips K, Wielgus et al (2011c) Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: implications for progression of Parkinson's disease. Neurotox Res 19:63–72

Zhang Z, Zhang K, Du X et al (2012) Neuroprotection of desferrioxamine in lipopolysaccharide-induced nigrostriatal dopamine neuron degeneration. Mol Med Rep 5(2):562–566

Zhou H, Falkenburger BH, Schulz JB et al (2007a) Silencing of the Pink1 gene expression by conditional RNAi does not induce dopaminergic neuron death in mice. Int J Biol Sci 3:242–250

Zhou F, Wu JY, Sun XL et al (2007b) Iptakalim alleviates rotenone-induced degeneration of dopaminergic neurons through inhibiting microglia-mediated neuroinflammation. Neuropsychopharmacology 32(12):2570–2580

Zhou H, Huang C, Tong J et al (2011) Temporal expression of mutant LRRK2 in adult rats impairs dopamine reuptake. Int J Biol Sci 7(6):753–761

Zhou F, Yao HH, Wu JY et al (2008) Opening of microglial K(ATP) channels inhibits rotenone-induced neuroinflammation. J Cell Mol Med 12(5A):1559–1570

Zimprich A, Biskup S, Leitner P et al (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44(4):601–607

Viral Vector-Based Models of Parkinson's Disease

Anke Van der Perren, Chris Van den Haute and Veerle Baekelandt

Abstract In order to study the molecular pathways of Parkinson's disease (PD) and to develop novel therapeutic strategies, scientific investigators rely on animal models. The identification of PD-associated genes has led to the development of genetic PD models as an alternative to toxin-based models. Viral vector-mediated loco-regional gene delivery provides an attractive way to express transgenes in the central nervous system. Several vector systems based on various viruses have been developed. In this chapter, we give an overview of the different viral vector systems used for targeting the CNS. Further, we describe the different viral vector-based PD models currently available based on overexpression strategies for autosomal dominant genes such as α-synuclein and LRRK2, and knockout or knockdown strategies for autosomal recessive genes, such as parkin, DJ-1, and PINK1. Models based on overexpression of α-synuclein are the most prevalent and extensively studied, and therefore the main focus of this chapter. Many efforts have been made to increase the expression levels of α-synuclein in the dopaminergic neurons. The best α-synuclein models currently available have been developed from a combined approach using newer AAV serotypes and optimized vector constructs, production, and purification methods. These third-generation α-synuclein models show improved face and predictive validity, and therefore offer the possibility to reliably test novel therapeutics.

Keywords Animal models · Parkinson's disease · Lentiviral vectors · Adeno-associated viral vectors · α-Synuclein

A. Van der Perren (✉) · C. Van den Haute · V. Baekelandt
Katholieke Universiteit Leuven, Leuven, Belgium
e-mail: Anke.VanderPerren@med.kuleuven.be

V. Baekelandt
e-mail: Veerle.Baekelandt@med.kuleuven.be

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1 Introduction

Parkinson's disease (PD) is the second most common age-related movement disorder. It is estimated that PD affects 1 % of the population at 65 years of age, which increases to 4–5 % in 85-year olds. To date, more than 4.5 million people suffer from PD worldwide, and this number is expected to double to 9 million in the next 20 years due to the improved living conditions and the increased life expectancy (Wirdefeldt et al. 2011). PD is a slowly progressing disorder that affects the dopaminergic neurons (DN) of the substantia nigra pars compacta (SNpc), a small nucleus in the midbrain. The loss of dopaminergic innervation leads to hyperactivity of the subthalamic nucleus and the globus pallidus, resulting in the progressive decline of movement control, which closely correlates with the degree of SNpc cell loss (Ma et al. 1997). At the onset of the motor symptoms, the striatal dopamine (DA) levels are already depleted by 80 % and approximately 50–70 % of the DN in the SNpc has been lost (Dauer and Przedborski 2003). However, the traditional view that the neuropathology of PD is restricted to nigral degeneration has been abandoned. For instance, Braak and colleagues define a six-stage pathological process where PD pathology starts in the olfactory bulb and the dorsal motor nucleus of the vagal nerve and extends to the midbrain and other brainstem regions in the later stages of the disease process (Braak et al. 2003; Hawkes et al. 2010). Next to the selective loss of the DN in the SN, PD is characterized by the presence of Lewy bodies (LBs) and Lewy neurites (LNs) in the surviving neurons. These cytoplasmic aggregates are predominantly composed of fibrillar forms of the protein α-synuclein, and can be found in several affected brain regions of most PD patients (Spillantini et al. 1997; Shults 2006). Currently, most treatments of PD are focused on the symptomatic improvement of motor symptoms related to the loss of the DN in the SNpc. These symptomatic treatments (pharmacological and surgical) significantly reduce PD-associated motor symptoms and improve quality of life and life expectancy. Still, the development of therapeutic approaches that slow or halt the disease progression, as well as the identification of reliable biomarkers for early diagnosis remains crucial.

Historically, PD has been considered a purely sporadic disorder without a clear etiology. Environmental factors (e.g., pesticides exposure, living in a rural area, and well water consumption) have been proposed as important risk factors or triggers for PD in the context of an aging brain. However, in the past decade, genetic studies of PD families from different geographical regions worldwide have strengthened the hypothesis that PD has a substantial genetic compound. The first gene linked to PD (SNCA, encoding for α-synuclein) was discovered through analysis of a large multigenerational Greek/Italian family, in which PD segregated in an autosomal dominant pattern (Polymeropoulos et al. 1997). Since then, 18 PD loci have been nominated through linkage analysis (PARK 1–15) or genome-wide association studies (PARK 16–18) (Farrer 2006; Belin and Westerlund 2008; Satake et al. 2009; Simon-Sanchez et al. 2009; Pankratz et al. 2009; Hamza et al. 2010). Mutations within the genes of six of these loci (SNCA, LRRK2, PRKN, PINK1, DJ-1, and ATP13A2) have conclusively been demonstrated to cause familial PD (Lesage and Brice 2009). In addition, common polymorphisms within two of the same genes (SNCA and LRRK2) are well validated risk factors for PD (Paisan-Ruiz 2009; Edwards et al. 2010). Although these familial forms of PD account for only 5–10 % of all PD patients, unraveling of the molecular pathways underlying familial forms of PD will greatly contribute to our understanding of sporadic PD since both forms share clinical and neuropathological features.

To study the molecular pathways of PD and to develop novel therapeutic strategies, investigators rely on animal models. However, to reliably translate the results from animal experiments to the human disease, one has to consider "the validity" of the animal model being used. In general, three criteria are taken into consideration: The face, predictive, and construct validity. The "face validity" refers to a similar pathophysiology; do the symptoms and pathology observed in the animal model resemble the ones seen in human patients? Face validity can sometimes be distracting because behavioral manifestations in rodents and people might differ. The "predictive validity" suggests that a treatment that is effective in the model will also be successful in patients. A model based on an established cause of the disease has "construct validity" and is useful in the understanding of the pathophysiological mechanism. A perfect animal model for PD should display age-dependent and progressive degeneration of DN, the cardinal motor symptoms of PD as well as nonmotor dysfunctions and LB pathology in surviving nigral cells and eventually other brain regions (high face validity). Furthermore, it should be responsive to dopamine replacement strategies (high predictive validity) and would be based on a known cause of the disease, e.g., genetic mutation causing familial PD (high construct validity). Although such a perfect PD model does not exist, significant progress has been made over the last 15 years. Currently, PD models are based on toxicological, transgenic, or viral vector-based stereotactic gene delivery approaches.

2 Toxin-Based Animal Models

Toxin models are the classical and the oldest experimental PD models. They aim at reproducing the pathological and behavioral changes of the human disease in rodents or primates by using neurotoxins (e.g., 6-OHDA, MPTP), which selectively accumulate in the substantia nigra DN, causing cellular dysfunction and cell death (Ungerstedt and Arbuthnott 1970; Betarbet et al. 2002). These toxins can be administered either locally or systemically depending on the type of toxin used and the species involved (Bezard and Przedborski 2011). The unilateral 6-OHDA rat model has been used extensively as a preclinical model to assess the antiparkinsonian effects of new pharmacological treatments (Jiang et al. 1993; Ilijic et al. 2011; Bjorklund et al. 2002; Kirik et al. 2002). Studies using MPTP have led to concepts such as "environmental toxicity" as a potential cause of dysfunction in sporadic PD and "mitochondrial dysfunction" as a potential pathogenic mechanism (Fox and Brotchie 2010). However, a major limitation of these toxin models is that they induce rather acute effects, which differ significantly from the slowly progressive pathology of human PD. Moreover, LB pathology is not present in the surviving neurons, and no other brain areas involved in PD are affected. Later, a variety of other toxic compounds (pesticides) like rotenone, paraquat, and maneb were tested in vivo in an attempt to find additional and improved toxin models (Hoglinger et al. 2006; Thiruchelvam et al. 2000). Interestingly, ubiquitine-positive LB-like inclusions were observed in the surviving nigral neurons of rotenone-treated animals (Betarbet et al. 2000). Despite these positive features, the substantial variability observed in all of these pesticide models limits their use for therapeutical development.

3 Transgenic Mouse Models

The discovery of α-synuclein as the first PARK gene in 1997 led to the development of the first genetic PD models. The A53T mutation in α-synuclein was identified first in a Greek/Italian family (Polymeropoulos et al. 1997). Later, the A30P mutation and later the E46K mutation were identified in a German and Spanish family (Kruger et al. 1998; Zarranz et al. 2004). Shortly after these findings, it was realized that an aggregated form of α-synuclein forms the main constituent of LBs in both sporadic and familial PD cases (Spillantini et al. 1997). In addition, duplications and triplications of the entire gene locus have been reported in different families (Chartier-Harlin et al. 2004; Ibanez et al. 2004; Singleton et al. 2003). Interestingly, gene triplication leads to earlier onset and faster progression of disease than duplication, indicating that disease severity is dependent on α-synuclein expression levels. Altogether, these findings provide strong evidence that α-synuclein plays an important role in the pathogenesis of PD. For these reasons, it was hypothesized that overexpression of α-synuclein in the

brain may lead to pathological features reminiscent of PD such as neuronal cell death and the accumulation of cellular inclusions. Many transgenic mice overexpressing human wild-type (WT) or mutant (A30P, A53T) α-synuclein have been generated over the last 10 years. The promoter driving α-synuclein expression has proven to be crucial in the development of the pathology. Other significant variations are the mouse strain, the presence or absence of endogenous α-synuclein, and whether the full length or the truncated form is expressed (detailed review by Magen and Chesselet 2010). Overexpression of both WT and several clinical mutants of human α-synuclein in transgenic mice have been shown to induce pathological accumulation of α-synuclein and neuronal dysfunction (Masliah et al. 2000; Freichel et al. 2007; Fleming et al. 2005; Kahle et al. 2001; Chesselet and Richter 2011). However, until now most transgenic α-synuclein mouse models failed to display clear dopaminergic cell loss and dopamine-dependent behavioral deficits. Some transgenic models expressing the double mutated (Thiruchelvam et al. 2004; Richfield et al. 2002; Chen et al. 2006) or truncated (Tofaris et al. 2006; Shelkovnikova et al. 2011; Wakamatsu et al. 2008) α-synuclein gene have reported dopaminergic cell loss, but the clinical relevance of these models is questionable.

Later, the identification of other PD-associated genes has led to the development of more genetic PD models. Overexpression strategies for autosomal dominant genes such as LRRK2 and knockout strategies for autosomal recessive genes such as Parkin, DJ-1, and PINK1 were used. Current transgenic LRRK2 mouse models are not very robust PD models. BAC transgenic mice expressing R1441G, G2019S LRRK2 present minimal evidence of neurodegeneration (Li et al. 2009, 2010). When the R1441C mutation is expressed under the control of endogenous regulatory elements, by knock-in of the R1441C mutation, no degeneration of DN is observed (Tong et al. 2009). For one R1441G-LRRK2 transgenic mouse abnormalities in the nigrostriatal system such as stimulated DA neurotransmission and L-dopa responsive behavioral defects have been described, but no DN loss was reported (Li et al. 2009). Recently, mice expressing the G2019S mutant showed loss of DN and dendrites at 19–20 months of age (Ramonet et al. 2011).

None of the parkin KO mice have substantial dopaminergic or behavioral abnormalities (Itier et al. 2003; Perez and Palmiter 2005; Von Coelln et al. 2004; Goldberg et al. 2003; Zhu et al. 2007). Some have subtle abnormalities in their DA neurotransmission or in the noradrenergic system of the locus coeruleus (Goldberg et al. 2003). Interestingly, overexpression of truncated human parkin (Q311X) led to progressive degeneration of DN (Lu et al. 2009), supporting the idea that some parkin mutants might act in a dominant negative fashion. Similar to the parkin KO mice, PINK-1 KO mice do not show any loss of DN, have normal levels of striatal DA and DA receptors are unchanged in most KO mice (Gautier et al. 2008; Kitada et al. 2007). Mild abnormalities in DA neurotransmission have been described (Kitada et al. 2007). DJ-1 KO mice do not exhibit any major abnormalities, and the number of DN and levels of striatal DA are unchanged. Abnormalities in DA neurotransmission in the nigrostriatal circuit and mitochondrial dysfunction were observed in some DJ-1 KO animals (Goldberg et al. 2005; Kim et al. 2005;

Andres-Mateos et al. 2007). To summarize, all parkin, PINK1, and DJ-1 KO animals present only a mild phenotype without clear neurodegeneration.

In conclusion, in some transgenic models based on mutations causing PD, loss of DN has been reported, specifically in mice expressing the double mutated (Thiruchelvam et al. 2004; Richfield et al. 2002; Chen et al. 2006) or truncated (Tofaris et al. 2006; Shelkovnikova et al. 2011; Wakamatsu et al. 2008) α-synuclein gene, a truncated form of parkin (Lu et al. 2009), or the G2019S mutation in LRRK2 (Ramonet et al. 2011). Nevertheless, most transgenic α-synuclein mouse models develop gradual α-synuclein pathology, but fail to display clear dopaminergic cell loss and dopamine-dependent behavioral deficits. This hurdle was overcome by direct targeting of the SN with viral vectors overexpressing PD-associated genes.

4 Viral Vector-Based PD Models

Viral vector-mediated loco-regional gene delivery provides an alternative way to express transgenes in the CNS with several advantages: Local transgene delivery allows for specific targeting of brain regions; transgene expression can be induced during adulthood, bypassing the risk of compensatory mechanisms during development; different doses can be applied; models can be created in multiple species and strains, ranging from rodents to nonhuman primates and finally different combinations of genes can easily be made. Since the first proof of principle of this technique, a continuously growing number of publications have proven the value of viral vector-mediated loco-regional transduction. Before we discuss the different viral vector-based PD models currently available, we will give an overview of the different viral vector systems used for delivery of transgenes to the brain.

4.1 Viral Vectors for Gene Transfer

Viruses are efficient vehicles to infect cells to introduce genetic material and force the cell to replicate the viral genome in order to produce new virus particles. Viruses can be engineered to nonreplicating viral vectors that retain their ability of entering cells and introducing genes. By deleting parts of the viral genome and replacing them by the genes of interest, application of the vector will result in a single-round infection without replication in the host cell. The general design of viral vectors is based on the physical separation in different plasmids of the *cis*-elements involved in the transfer of the vector genome from *trans*-acting elements encoding for the viral (structural) proteins. These plasmids deliver the necessary components to assemble a vector particle during the vector production in producer cells. Viral vectors can be used for both overexpression and silencing of certain genes. The transgene encoded by the vector can be a reporter protein for visualization of the

labeled cells by immunohistochemical or non-invasive imaging techniques such as bioluminescence, nuclear medicine techniques, or magnetic resonance imaging (Deroose et al. 2009). The expressed protein can be a therapeutic protein for gene therapy applications (Manfredsson et al. 2007; Vercammen et al. 2006; Winklhofer 2007) or as we will focus on in this chapter, a disease-related protein used for disease modeling (Kirik et al. 2002, 2003; Lauwers et al. 2003; Klein et al. 2002). Several vector systems based on various viruses have been developed. The choice of the vector system depends on the size of the gene of interest, the required duration of gene expression, the target cells and biosafety issues (see Table 1). For stable gene transfer in the brain, LV and rAAV vectors are the vector systems of choice since they lead to efficient and long-term gene expression in the rodent brain. The different vector systems will be described in more detail in the following section.

4.1.1 Lentiviral Vectors

LVs are derived from lentiviruses, a family of complex retroviruses that have the ability to replicate in non-dividing cells as opposed to simple retroviruses. Vectors derived from these viruses are an attractive means for gene transfer to the central nervous system (CNS) because they are capable of efficiently transducing non-dividing mature neurons. The prototypical lentivirus is the human immunodeficiency virus type 1 (HIV-1), which has a diameter of 110 nm and contains two copies of a 9200 bp ss RNA genome. The genome has three major coding regions in common with simple retroviruses (*gag*, *pol*, and *env*), flanked by two long terminal repeats (LTR). *Gag* encodes the structural core protein matrix, capsid, and nucleocapsid, *pol* encodes the viral enzymes [protease, reverse transcriptase (RT), and integrase] and *env* encodes the glycoprotein components of the viral envelope. In addition, complex retroviruses have six accessory genes (*tat, rev, vpr, vpu, vif*, and *nef*) that encode for additional regulatory proteins. These accessory genes are not essential for viral replication in cell culture, but determine the pathogenicity of viral infection in vivo. Several studies have shown that LVs derived from HIV-1 are indeed capable of efficient and stable expression of the transgene in vivo without induction of significant immune response (Baekelandt et al. 2002; Naldini et al. 1996a, b; Zufferey et al. 1997).

Design of Lentiviral Vectors

The first generation of LVs derived from HIV-1 was developed in 1996, by Naldini et al. (1996a, b). LVs are usually produced by triple transfection of HEK293T cells with three different plasmids, providing the *cis*- and *trans*-elements necessary for the production of vector particles (Fig. 1). The first plasmid, the packaging plasmid encodes structural viral proteins from *gag* and enzymes from *pol* under the control of a constitutive promoter. For biosafety reasons, the packaging signal (Ψ) and the *env* gene have been deleted. The envelope plasmid mostly codes for the envelope glycoprotein of the vesicular stomatitis virus (VSV-G). This VSV-G envelope

Table 1 Characteristics of gene transfer vectors [adapted from Baekelandt et al. (2000) with modifications]

	Retroviral vectors	Lentiviral vectors	Adenoviral vectors	Adeno-associated viral vectors	Herpes simplex type-1 vectors
Derived from	Oncoretro virus	Lentivirus	Adenovirus	Adeno-associated virus	Herpes simples virus
Genome	ss RNA	ss RNA	ds DNA	ss DNA	ds DNA
Envelop	Yes	Yes	No	No	Yes
Maximum insert size	7–7.5 kb	7–7.5 kb	30 kb	4.5–5 kb	50–150 kb
Integration	Yes	Yes	No	Yes/no	No
Stability of expression	Long (silencing)	Long	Short/ prolonged	Long	High, but low in latency phase
Immune response	Low	Low	Extensive/ reduced	Medium	Medium
Titers (viral particles/ ml)	10^8	10^8	10^{12}	10^{12}	10^{12}

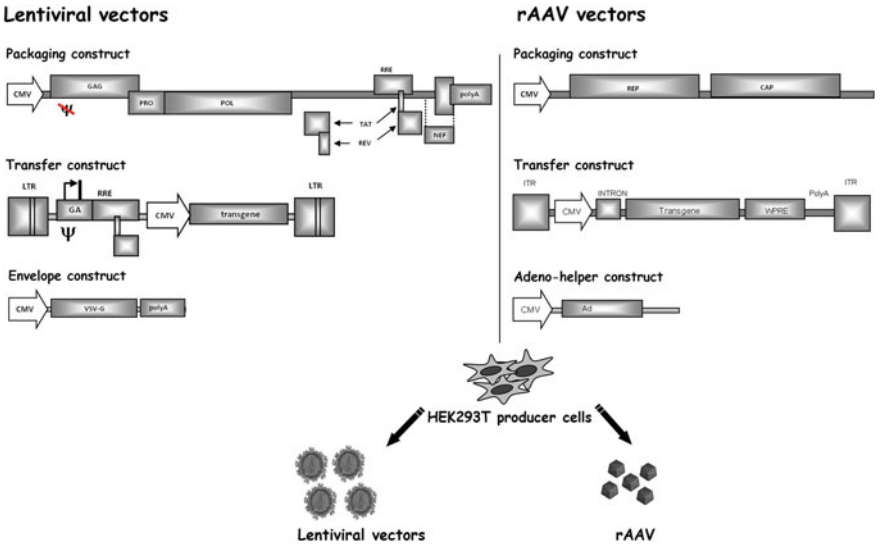

Fig. 1 Schematic representation of the different constructs essential for lentiviral vector (third generation) and rAAV vector production

results in a broad cellular tropism and efficiently targets neurons and astrocytes in the CNS and ensures a better stability of the vector particle compared to HIV enveloped vectors. The third plasmid, the transfer plasmid encodes the transgene of interest under the control of a heterologous promoter. It contains *cis*-acting sequences necessary for encapsidation, reverse transcription, and integration flanked by two LTRs. LTRs are necessary for integration of the proviral DNA mediated by the viral integrase and also contain a promoter for proviral DNA transcription. After triple transfection, the vector particles produced by the HEK293T cells are released into the medium and can be purified and concentrated.

After the initial validation of the LV system, additional elements were introduced to increase the specificity and enhance the efficiency of these vectors. A first improvement was the insertion of the central polypurine tract (cPPT), which is thought to facilitate reverse transcription and nuclear import of the lentiviral preintegration complex prior to vector integration. Inclusion of this element in the LV increased the transduction efficiency and gene expression 3- to 10-fold (De Rijck et al. 2005; Zennou et al. 2000). A second improvement was the introduction of the Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) that enhanced expression levels about 5- to 8-fold in a number of different cell lines and tissues due to an increased mRNA stability in the cytoplasm (Zufferey et al. 1999). LV containing both the cPPT and the WPRE has higher in vivo transduction efficiency than the sum of the expression levels reached by LV containing only the cPPT or the WPRE (Baekelandt et al. 2002).

Biosafety of Lentiviral Vectors

Lentiviral vectors derived from HIV-1 may provoke biosafety concerns because of the well-known pathogenicity of the parental virus. An important issue is the potential occurrence of replication competent retroviruses (RCR), which can theoretically be generated during vector production by homologous recombination. A lot of effort has been undertaken to improve the biosafety of lentiviral vectors. In addition to the removal of accessory genes in the transfer plasmid, thereby reducing or eliminating the pathogenicity of HIV-1, safer LVs have been developed by splitting the *cis*- and *trans*-acting viral sequences over separate expression plasmids and deleting promoter and enhancer elements in the transfer vector itself. This reduces the probability of homologous recombination.

In the first generation of vectors, the *env* gene coding for the natural HIV envelope was replaced by the VSG-G gene. As a result, no WT HIV-1 could ever arise even if the three plasmids recombined. The encapsidation signal was removed from the packaging plasmid and the viral LTRs were replaced by a heterologous promoter at the 5′-end and a polyadenylation signal at the 3′-end. In the second-generation vectors, the virulence genes *vpr, vpu, vif*, and *nef* were deleted from the packaging construct without loss of transduction capacity (Zufferey et al. 1997). Since it is known that WT HIV-1 loses its pathogenicity when devoid of these proteins, deletion of these genes further increased biosafety. Vectors from the third generation do not require *tat* anymore since the U3 region of the 5′-LTR, which

enhances viral transcription in the presence of tat was replaced by a constitutive promoter (Dull et al. 1998). This allowed *tat* gene deletion from the packaging plasmids. In addition, *rev*, an accessory protein mediating nuclear export of viral RNA was supplied from a fourth plasmid, further reducing the chance of recombination.

Another strategy to improve the biosafety of LVs involves deletion of the promoter and enhancer elements located in the 3′-LTR U3 region of the transfer plasmid. Vectors containing this deletion are called self-inactivating (SIN) vectors because the deletion is copied from the 3′-LTR to the 5′-LTR during reverse transcription, resulting in modified LTRs with only weak promoter activity (10 % of original activity). In addition, this deletion in the 3′-LTR prevents potential transcriptional activation of any (onco)gene upstream or downstream of the integration site. Moreover, SIN vectors cannot be rescued by WT HIV-1 (Bukovsky et al. 1999).

4.1.2 Recombinant Adeno-Associated Viral Vectors

Recombinant adeno-associated viral (rAAV) vectors derived from the adeno-associated virus (AAV) have been an attractive gene delivery vehicle since they were first engineered almost three decades ago (Samulski et al. 1982). The main reason for this is the unique combination of attractive properties: rAAV vectors ensure efficient gene transfer in different tissues. rAAV vectors are considered safe because the AAV virus they are derived from, is a non-pathogenic parvovirus whose replication depends on co-infection with a lytic helper virus, usually a member of the adenovirus (Atchison et al. 1965) or herpes virus family (Buller et al. 1981). rAAV vectors are also devoid of all WT AAV genes, making reversion to replication competent forms virtually impossible. In addition, compared to adenoviral vectors, the administration of rAAV vectors in vivo usually does not elicit a host immune response resulting in destruction of the transduced cells as shown in preclinical studies in small animal models.

rAAV vectors are capable of packaging gene cassettes of 4.5–5 kb (Baekelandt et al. 2000; Grieger and Samulski 2005). rAAV gene cassettes do not integrate into the host genome, but they readily persist for months to years in slowly dividing or nondividing cells. Indeed, studies have demonstrated that rAAV vectors can efficiently transduce a number of somatic tissues, including muscle (Xiao et al. 1996), liver (Miao et al. 1998), heart (Chu et al. 2004), retina (Hellstrom et al. 2009), and the CNS (Tenenbaum et al. 2004). To date, the most widely used rAAV vector is based on the AAV2 serotype, which has however been shown to contain some drawbacks. rAAV2 transduces neurons efficiently in the immediate vicinity of the injection site (Passini et al. 2004), but requires multiple injections, convection-enhanced delivery (Bankiewicz et al. 2000; Cunningham et al. 2000) or addition of agents such as mannitol, basic fibroblast growth factor (bFGF) or heparin to transduce larger brain volumes (Mastakov et al. 2001, 2002; Burger et al. 2005; Hadaczek et al. 2004). Furthermore, the clinical application of rAAV2/2 may be

limited by pre-existing immunity to AAV2, which is present in most humans (Moskalenko et al. 2000). Finally, rAAV2 mainly targets neurons, although other cell types in the CNS might also be transduced. The limitations of rAAV2 have advanced the evaluation of alternative/artificial serotypes with broader cellular targets, higher transduction efficiencies and potential to evade pre-existing immunity to the AAV2 capsid (Paterna et al. 2004; Burger et al. 2004; Taymans et al. 2007; McFarland et al. 2009a; Dodiya et al. 2009).

A possible route to find the ideal rAAV tropism, next to modification of the rAAV capsid structure by chemical, immunological, or genetic means (Rabinowitz and Samulski 2000), is exploiting the natural capsid diversity of newly isolated serotypes by packaging rAAV2 genomes into capsids derived from other human or non-human AAV isolates (Grimm and Kay 2003). To this end, up until now, most researchers employ hybrid *trans*-complementing constructs that encode *rep* from AAV2, whereas *cap* is derived from the serotype displaying the cell tropism of choice. These hybrid vectors are therefore indicated as rAAV2/5, rAAV2/7, etc., where the second number refers to the capsid serotype. Among the more than 120 identified AAV variants that have been isolated from adenovirus stock or from human/non-human primate tissues, AAV2/1 to AAV2/10 are currently being developed as recombinant vectors for brain applications (Grimm and Kay 2003; Rutledge et al. 1998; Gao et al. 2002, 2003, 2004, 2005; Schmidt et al. 2006; Wu et al. 2006). This major advance in rAAV vectorology has significantly broadened potential applications of rAAV vectors for clinical gene therapy or disease modeling, and offers more options for the selection of a suitable rAAV variant per specific application. Recombinant AAV vectors are currently considered as a first choice option for brain applications both to generate preclinical models of neurodegeneration and for gene therapy. Indeed, rAAV vectors have proven useful to model diseases such as PD and have also been tested in various phases of clinical development of gene therapy for PD (Christine et al. 2009; Kaplitt et al. 2007) and Alzheimer's disease (Mandel 2010).

Design of rAAV Vectors

Historically, most recombinant AAV vectors were based on serotype 2 (rAAV2/2) that constitutes the prototype of the genus, and was produced by means of two plasmids (the transfer and the packaging plasmid) and an infectious adenovirus. The transfer plasmid carries a transgene expression cassette flanked by the AAV2 inverted terminal repeats (ITRs), which are the only *cis*-acting elements required for replication and packaging of the recombinant genome (Samulski et al. 1987). The ends of the AAV2 genome consist of a 145 nucleotide-long ITR that, due to the multipalindromic nature of the terminal 125 bases, can fold on itself via complementary base pairing and form a characteristic T-shaped hairpin structure. The AAV2 nonstructural (rep) gene and a specific structural (cap) gene that depends on the serotype used are supplied in trans on the second plasmid, the so-called packaging plasmid. The adenoviral (Ad) helper functions were originally supplied by infection of rAAV producer cells with a WT adenovirus. The finding

that Ad helper functions are provided by expression of E1A, E1B, E2A, E4ORF6, and VA RNAs, enabled subsequent Ad-free production of rAAV vector stocks by incorporating these sequences into a plasmid (referred to as adeno helper plasmid) and transfecting it together with the two above-mentioned plasmids. Upon introduction of all these constructs into the producer cells, vector particles are generated (Fig. 1).

Extensive efforts have been focused on developing versatile and scalable manufacturing processes for rAAV vector production with attention to compatibility with good manufacturing practice (GMP) (Gao et al. 2000; Clark et al. 1995; Blouin et al. 2004; Wright 2009). In our research group, we optimized a scalable and flexible serum-free rAAV vector production system, allowing a swift adaptation for production of different serotypes (Lock et al. 2010; Van der Perren et al. 2011, 2014; Toelen et al. 2014).

4.1.3 Adenoviral Vectors

Adenoviral vectors are derived from adenoviruses, DNA viruses with a linear double- stranded genome of 36 kb. The viral genome encodes for about 50 different proteins, 11 of which are structural and used to build the virion. These viruses have been isolated from a large number of species, and in humans, they primarily infect the respiratory airways and the gut causing mild respiratory and gastroenteric diseases. More than 40 AdV serotypes have been described, some more widespread than others (Davison et al. 2003). Because of their low pathogenicity and wide tropism, AdV-based vectors could be good candidates for gene delivery transfer. Unfortunately, the broad pre-existing immunity in the population prevents the use of vectors derived from the most common serotypes. Furthermore, the high immunogenicity of AdV proteins severely limits re-administration. To bypass these problems, AdV are mostly derived from rare serotypes, usually AdV2 or 5. AdV proved to be able to transduce a great variety of post-mitotic cells in the tissues like lung, skeletal muscle, heart, and brain (Howarth et al. 2010).

Design of Adenoviral Vectors

In first-generation AdV vectors, the early gene 1A (E1A), a regulatory gene essential for replication, was deleted (Graham and Prevec 1995). To further reduce the risk of the occurrence of a replication competent virus, E1B and E3, genes that play a role in modulating AdV-specific immunity, as well as E2 and E4 were additionally deleted (Campos and Barry 2007). This resulted in a vector system with a capacity to introduce up to 30 kb of DNA. To produce vector particles, these deleted genes essential for replication are provided by a helper virus or DNA construct that provides the missing functions in trans. Vector particles are generated by transfection of the vector construct and a helper virus, or by double transfection with a packaging construct. Alternatively, AdV vectors can also be produced using a packaging cell line, which stably expresses the structural and

regulatory proteins required for vector assembly (Wang et al. 2009). Efforts are being made to circumvent the broad pre-existing immunity, which currently limits the use of this vector type.

4.1.4 Herpes Simplex Vectors

Herpes virus vectors are mainly derived from HSV type1, a very large double-stranded DNA virus of 152 kb. The viral genome encodes for more than 80 different proteins, which can be divided into essential and nonessential genes whether they are required for viral replication. HSV-1 is spread by contact, infects, and replicates in the skin or mucous membranes, and is taken up by sensory nerve terminals where it establishes a latent state, from which the virus can subsequently be reactivated and spread to other individuals. HSV-1 is endemic and more than 70 % of the people have a specific immune response that is maintained active due to intermittent reactivation of the infection. The main limitation in the use of HSV-1 vectors is the pre-existing immunity in humans, eliminating vector particles and transduced cells exposing HSV proteins on their surface. A second safety concern is the presence of latently infected cells, which may, upon transduction, offer a suitable environment for the HSV vector to recombine with the WT genome. These limitations have severely limited the range of applications of HSV vectors. On the other hand, these limitations can be considered as an advantage when using them as oncolytic vectors, targeting proliferating tumor cells.

Design of Herpes Simplex Vectors

The HSV-1 genome contains a significant portion of viral genes that are considered "non-essential," which have been removed during vector construction. This elimination makes room for up to 50–150 kb of DNA depending on the type of HSV vector, making them the largest carriers among all viral vectors. The genetic complexibility of the virus genome allows the production of different types of vectors with different properties. Currently, three classes of vectors are derived from HSV-1: Conditionally replication competent vectors, replication incompetent vectors, and amplicon-based vectors (de Silva and Bowers 2009). The first class is designed by deleting the genes not essential for replication, but important for pathogenicity. These vectors are capable of replicating only in certain cell types and tissues in vivo, and are typically used in the development of therapies for malignant brain tumors (Markert et al. 2000). They are referred as oncolytic HSV-1 vectors. For the second class, the replication incompetent vectors, one or more immediate-early genes essential for lytic replication and reactivation have been deleted, but they retain the ability to establish latency. For the production of vector particles, this genetic information is provided in trans by a replication competent HSV strain, a packaging construct or by a specific packaging cell line that stable expresses the required proteins. These replication incompetent vectors have been used for preclinical studies of neurodegenerative diseases and chronic pain (Burton et al. 2002). To generate the third class, the amplicon-based vectors, a single origin

of replication and a single packaging/cleavage signal from the WT HSV-1 was incorporated into a standard bacterial plasmid, called the amplicon. A transgene can be cloned into the amplicon plasmid and defective viral vector particles can be produced by complementing the necessary genes by a defective helper virus, a packaging construct, or specific packaging cells (de Silva and Bowers 2009). This class is the safest as it carries minimal viral sequences, but requires optimization to increase vector titers.

4.2 From Viral Vector to Animal Models

4.2.1 Viral Vector-Based α-Synuclein Overexpression Models

Overexpression of α-synuclein by direct targeting of the SN of rats, mice, or non-human primates with viral vectors offers a valuable alternative approach to the α-synuclein transgenic mice. Using viral vectors, high transgene expression levels can be achieved, which might be crucial since the disease onset and severity depends on the level of α-synuclein expression. Two vector systems have been explored for this purpose: rAAV and LV (Table 2).

The viral vector approach was initially explored almost simultaneously by a number of different groups using either rAAV2/2 or LV vectors (Kirik et al. 2002; Klein et al. 2002; Lauwers et al. 2003; Lo Bianco et al. 2002). In rats injection of rAAV2/2 vectors expressing either WT, A30P, or A53T mutant human α-synuclein unilaterally into the SN (Kirik et al. 2002; Klein et al. 2002) induced efficient expression of α-synuclein in nigral DN, accompanied by cellular and axonal pathologies and nigral DN loss that developed progressively over time. These first-generation rAAV2/2-based α-synuclein models displayed progressive neurodegeneration, but the loss of the nigral TH-positive neurons (25–80 %) as well as the time course described was quite variable (6 weeks up to 1 year) (Kirik et al. 2002; Klein et al. 2002). LV vectors encoding WT, A30P, or A53T mutant α-synuclein were also capable of inducing neuronal cell loss in rats, but less pronounced and more delayed (24–35 % cell loss at 5 months) (Lo Bianco et al. 2002). Dystrophic neurites and swollen perikarya were detected in the remaining DNs and cytoplasmic accumulations of α-synuclein were found in cell bodies as well as in neurites. Many attempts were made to reproduce and improve these rat models using rAAV2/2 (Yamada et al. 2004; Maingay et al. 2006; Mochizuki et al. 2006; Chung et al. 2009) or LV (Lauwers et al. 2007), but similar results were obtained.

Both LV and rAAV vectors have also been used in mice. Work from our own group showed that injection of LV-WT and A30P α-synuclein in different mouse brain regions (striatum, amygdala, SN) induced time-dependent neuropathological changes including neuritic enlargements and cytoplasmic inclusions. Furthermore, nigral overexpression of A30P α-synuclein resulted in a 10–25 % cell loss at 10–12 months (Lauwers et al. 2003). In another study, nigral injection of rAAV2/2 WT α-synuclein induced a 25 % cell loss at 24 weeks; however, no obvious

Table 2 Human α-synuclein viral vector-based rodent models

α-Synuclein	Vector system	Promoter	Species	DN loss in SN	Time course	α-SYN aggregates	Ubiq.	phos. S129	Motor phenotype	L-dopa reversible
First generation										
WT, A53T (Kirik et al. 2002)	rAAV2/2	CBA	Rat	30–80 %	8–16 weeks	Yes	–	–	Mild	–
A30P (Klein et al. 2002)	rAAV2/2	CBA	Rat	53 %	1 year	Yes	–	–	–	–
WT, A30P, A53T (Lo Bianco et al. 2002)	LV	PGK	Rat	24–35 %	20 weeks	Yes	No	–	–	–
WT, A30P (Lauwers et al. 2003)	LV	CMV	Mice	10–25 %	40–52 weeks	Yes	Yes	–	–	–
WT, A53T (Kirik et al. 2003)	rAAV2/2	CBA	Prim.	30–60 %	16 weeks	Yes	–	–	Mild	–
WT (Yamada et al. 2004)	rAAV2/2	ND	Rat	50 %	13 weeks	Yes	No	Yes	No/mild	–
A53T (Maingay et al. 2006)	rAAV2/2	CBA	Rat	±40 %	14 weeks	–	–	–	–	–
A30P (Lauwers et al. 2007)	LV	CMV	Rat	21–52 %	46 weeks	Yes	Yes	–	Mild	Yes
WT (St Martin et al. 2007; Theodore et al. 2008)	rAAV2/2	CBA	Mice	25 %	24 weeks	No	–	–	–	–
A53T (Chung et al. 2009)	rAAV2/2	Syn-1	Rat	35 %	17 weeks	–	–	–	–	–
Second generation										
WT, A53T (Eslamboli et al. 2007)	rAAV2/5	CBA	Prim.	A53T 40 %	1 year	Yes Θ	Yes	Yes	Moderate	–
WT, S129D/A (Gorbatyuk et al. 2008)	rAAV2/5	CBA	Rat	WT 60 % S129A 70 %	26 weeks 4 weeks	Yes	–	–	–	–
WT, A30P, S129D/A (Azeredo da Silveira et al. 2009)	rAAV2/6	CMV	Rat	WT, A30P 20 % S129A 70 %	8 weeks	Yes Θ	–	Yes	–	–

(continued)

Table 2 (continued)

α-Synuclein	Vector system	Promoter	Species	DN loss in SN	Time course	α-SYN aggregates	Ubiq.	phos. S129	Motor phenotype	L-dopa reversible
WT, S129D/A (McFarland et al. 2009a)	rAAV2/8	CBA	rat	26 %	6 weeks	yes	yes	–	–	–
A53T (Koprich et al. 2011)	rAAV1/2	CBA	rat	28 %	6 weeks	yes Θ	–	–	mild	–
WT, A53T, A30P (Winner et al. 2011), E46K, E57K*, E35K*	LV	CMV	rat	17–40 %	3 weeks	–	–	–	–	–
Third generation										
WT (Decressac et al. 2012)	rAAV2/6	Syn-1	rat	80 %	6–16 weeks	yes	–	yes	good	yes
A53T (Van der Perren et al. 2014)	rAAV2/7	CMV/Syn-1	rat	80–90 %	4 weeks	yes	yes		good	yes
A53T, WT (Oliveras-Salvá et al. 2013)	rAAV2/7	CMV/Syn-1	mice	82 %	8 weeks	yes	–	yes	–	–

Ubiq ubiquitination; *posph.S129* S129 α-synuclein phosphorylation; *ND* not described; *prim* primates; – not determined; * oligomeric mutants; Θ proteinase K-resistant aggregates; *CBA* chicken β-actin promoter; *PGK* phosphoglycerate kinase promoter; *CMV* cytomegalovirus promoter; *Syn-1* synapsin-1 promoter; *CMV/Syn1* CMV enhanced synapsin 1 promoter

cytoplasmic inclusions were detected in the α-synuclein expressing cells (St Martin et al. 2007). Overall, the pathology observed in mice, both the onset and the severity, appeared less severe compared to the described rat models. rAAV vectors were also applied in non-human primates. Sixteen weeks after nigral injection of rAAV2/2 WT or A53T α-synuclein, 30–60 % of the nigral cells were lost, which resulted in mild motor deficits (Kirik et al. 2003).

The limitations of rAAV2/2 have prompted the evaluation of alternative serotypes with broader cellular targets and higher transduction efficiencies. As a result, other serotypes, notably rAAV2/5, rAAV2/6, rAAV2/8, and rAAV1/2, have been used in rats and non-human primates to overexpress α-synuclein (Eslamboli et al. 2007; Gorbatyuk et al. 2008; Azeredo da Silveira et al. 2009; Sanchez-Guajardo et al. 2010; Koprich et al. 2011; McFarland et al. 2009a). Since phosphorylation of α-synuclein at serine 129 is a common feature in patients suffering of synucleinopathies, mutations mimicking α-synuclein phosphorylation might also enhance dopaminergic pathology. Mutations mimicking (S129D) or preventing phosphorylation (S129A) have been expressed using rAAV2/5, rAAV2/6, and rAAV2/8 in the rat SN (Gorbatyuk et al. 2008; Azeredo da Silveira et al. 2009; McFarland et al. 2009b). Interestingly, in the studies using rAAV2/5 and rAAV2/6, the S129A mutation, which can no longer be phosphorylated, showed enhanced toxicity (70 % cell loss at 4 weeks) compared to WT (60 % cell loss at 26 weeks), while the S129D mutant showed reduced or no toxicity (Gorbatyuk et al. 2008; Azeredo da Silveira et al. 2009). With rAAV2/8, the α-synuclein mediated toxicity was equivalent for WT as well as both S129 mutants (26 % at 6 weeks) (McFarland et al. 2009b), an observation potentially explained by different expression levels obtained by rAAV2/8 compared to rAAV2/5 or rAAV2/6 in the substantia nigra (Van der Perren et al. 2011). rAAV1/2 (chimeric vector) driven overexpression of A53T α-synuclein in the SN of rats caused a dopaminergic cell loss of 28 % at 6 weeks (Koprich et al. 2011). In this study, the choice of the vector titer was crucial since too high vector titers caused unspecific toxic effects. These second-generation rAAV-based α-synuclein rat models display a more reproducible neurodegeneration (15–60 % loss 6–26 weeks p.i.) (Gorbatyuk et al. 2008; Azeredo da Silveira et al. 2009; Sanchez-Guajardo et al. 2010; Koprich et al. 2011). However, even in these rat models, the time course and loss of the nigral TH-positive neurons still suffered from variability, which hindered clear motor deficits.

Different species of α-synuclein could also influence the levels of pathology. Winner et al. evaluated two artificial mutants of α-synuclein (E57K, E35K) with the propensity to form oligomeric aggregates and one faster fibril forming mutant of α-synuclein (α-SYN 30-110) in vivo (Winner et al. 2011). E57K and E35K mutant α-synuclein were delivered in the rat SN using LV and resulted in a dopaminergic loss of, respectively, 51 % and 50 %, which turned out to be more toxic than WT (32 %), A30P (38 %), E46K (40 %), and A53T (17 %) α-synuclein at 3 weeks p.i. Remarkably, the faster fibril forming mutant A53T did not show a significant decrease in dopaminergic cell number. These data further support the idea that the oligomeric species and not the fibrillar form of synuclein are toxic and

contribute to dopaminergic degeneration. Although being very instrumental, the dopaminergic cell loss of 17–50 % in these LV-based rat models is insufficient for behavioral manifestations, which require at least 60–70 % cell loss. In non-human primates, better results were obtained. Overexpression of WT or A53T mutant α-synuclein using rAAV2/5 resulted in a clear difference at the behavioral and morphological level (Eslamboli et al. 2007). Stronger motor impairments were observed in the A53T group compared to the WT group 42 weeks after injection. After 1 year, TH-positive fiber loss was observed in the striatum of both groups, but it was more pronounced in the A53T group. In both groups, the remaining fibers were dystrophic and contained α-synuclein positive insoluble inclusions. Many α-synuclein positive aggregates were found to be phosphorylated at serine 129. When analyzing the SN, a loss of up to 40 % of the DNs was observed in the A53T α-synuclein animals in contrast to WT α-synuclein animals, which only differed in two out of eight animals from the eGFP control animals. Furthermore, at 1 year p.i. ubiquitin positive inclusions were detected in the surviving nigral neurons of both groups.

To summarize, viral vector-mediated α-synuclein models display synuclein pathology and clear dopaminergic neurodegeneration in contrast to α-synuclein transgenic mice. The transgene expression in DN achieved with the novel rAAV serotypes (rAAV2/5, 2/6, 2/8, and 2/1) is improved compared to rAAV2/2 and substantially higher compared to LV probably due to their high titers and tropism for DN neurons, resulting in higher levels of neurodegeneration. Although promising, these models still suffer from a certain degree of variability, a slow progression of the phenotype and lack overt behavioral impairments, hindering their usefulness for testing novel therapeutics.

To address these issues, a third generation of α-synuclein models has been developed by optimizing the promoter, the rAAV serotype, the vector construct, as well as the vector titer and purity of the vector preparation. Decressac et al. reported progressive dopaminergic neurodegeneration up to 75 % at 8–16 weeks after nigral injection of rAAV2/6 using the neuron specific synapsin-1 promoter to drive the expression of WT α-synuclein (Decressac et al. 2012). The cell loss was preceded by degenerative changes of striatal axons and terminals, and the presence of α-synuclein positive inclusions in dystrophic axons and dendrites. This high level of neurodegeneration resulted in clear behavioral deficits seen in the cylinder test and the amphetamine-induced rotation test. Our group has recently shown that rAAV2/7 outperformed most serotypes in terms of transduction efficiency and expression levels in rat SN (Van der Perren et al. 2011). Based on these data, we developed a rat model for PD by injection of rAAV2/7 encoding A53T α-synuclein into the SN (Fig. 2) (Van der Perren et al. 2014). High α-synuclein expression was observed four days post-injection. At least 90 % of the DNs were transduced, which resulted in progressive nigrostriatal pathology up to 80 % and behavioral deficits in 4 weeks' time period. Levodopa (L-dopa) was found to reverse the behavioral phenotype. Non-invasive PET and MR imaging allowed longitudinal monitoring of neurodegeneration. In addition, ubiquitin positive α-synuclein aggregates were detected. This rat PD model successfully recapitulates the

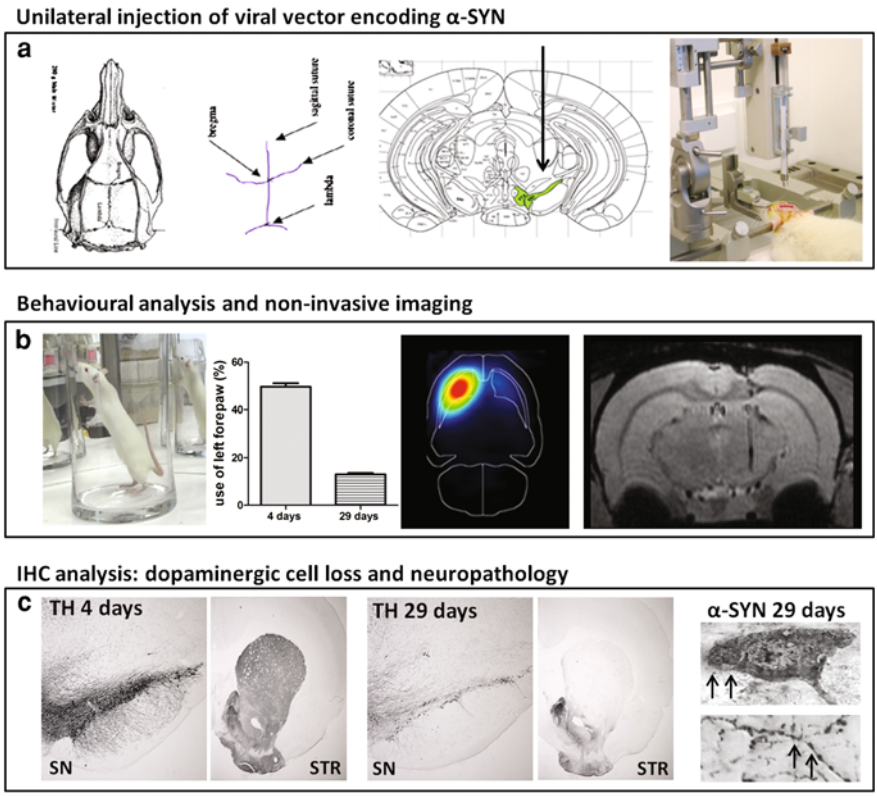

Fig. 2 In vivo validation of an rAAV vector encoding α-synuclein using behavioral, PET, MRI, and IHC analysis. (**a**) Stereotactic injection of rAAV vector encoding α-synuclein (α-SYN) in the rat SN. (**b**) Cylinder test, PET imaging (dopamine transporter), and MR imaging. (**c**) Immunohistochemical staining for tyrosine hydroxylase (TH) in the substantia nigra (SN) and the striatum (STR) 4 and 29 days after vector injection in the SN, and for α-synuclein 29 days after injection

progressive degeneration of DN and associated motor deficits together with the formation of α-synuclein inclusions. In parallel to this study, we also showed that strong and significant α-synuclein-induced neuropathology and progressive dopaminergic neurodegeneration can be achieved in mouse brain by means of rAAV2/7 (Oliveras-Salva et al. 2013). We noted a significant and dose-dependent α-synucleinopathy over time upon nigral viral vector-mediated α-synuclein overexpression. We obtained a strong, progressive, and dose-dependent loss of DNs in the SN, reaching a maximum of 82 % after 8 weeks. When comparing wild-type to A53T mutant α-synuclein at the same vector dose, both induced a similar degree of dopaminergic cell death (Oliveras-Salva et al. 2013). These major improvements can probably be explained by the choice of the rAAV7 serotype, the use of the CMV enhanced synapsin-1 promoter, and the vector titer combined with an optimized vector preparation. We believe that these improved rodent models will be of great value for further development and testing of neuroprotective strategies.

4.2.2 Viral Vector-Based LRRK2 Overexpression Models

rAAV are not suitable for the delivery of the 7581 bp LRRK2 cDNA to the nigrostriatal system due to their limited packaging capacity. Even for LV, the size of the LRRK2 cDNA is approaching the packaging limitations. Our group has succeeded to generate functional LRRK2 LV (Civiero et al. 2012), however, with reduced titers, which compromises the chances to induce phenotypic effects in vivo. To date, two groups have reported the generation of viral vector-mediated LRRK2 PD models using either recombinant adenoviral (rAdv) or herpes simplex vectors (HSV) (Table 3) (Dusonchet et al. 2011; Lee et al. 2010). rAd vectors expressing WT or G2019S mutant LRRK2 were injected into six sites within the rat striatum and nigral transduction relied on retrograde transport of the vector. Quantification of the expression levels revealed that 31 % of the nigral DN were transduced. Injection of Adv encoding mutant G2019S LRRK2 resulted in progressive loss of nigral DN up to 10 % after 10 days and 21 % after 42 days. WT LRRK2 rAdv did not induce any degeneration. No abnormal α-synuclein accumulation, ubiquitinated, or phosphorylated aggregates were found in WT or G2019S LRRK2 expressing neurons in the SN (Dusonchet et al. 2011). Lee et al. generated an LRRK2 mouse model using HSV vectors (Lee et al. 2010). HSV vectors encoding WT LRRK2, G2019S, or G2019S/D1994A LRRK2 were injected into the mouse striatum resulting in a retrograde transduction of 75 % of the nigral neurons. Three weeks after injection, overexpression of G2019S LRRK2 induced a progressive loss of 50 % of the nigral DN. G2019S-D1994A (kinase dead) LRRK2 caused no neuronal loss, similar to WT LRRK2 and GFP control vectors. These data link LRRK2 toxicity to elevated kinase activity in vivo.

4.2.3 Viral Vector-Based Parkin, DJ-1, and PINK1 Knockdown Models

Since parkin, PINK1, and DJ-1 KO mice do not display a clear phenotype, viral vector-mediated gene silencing in the adult brain could be explored as an alternative strategy. Stable knockdown of gene expression can be achieved by viral vector-mediated RNA interference after stereotactic injection into the brain (Ulusoy et al. 2009). Adult knockdown with viral vector technology has not been published for parkin or DJ-1. Haque et al. achieved a shRNA-mediated knockdown of PINK1 of 71 % in the striatum and 68 % in the SN by stereotactic striatal injection using an adenoviral vector in adult rats (Haque et al. 2012). This knockdown of PINK1 in the SN increased the sensitivity to MPTP administration, resulting in an increased loss of DN in the SN and terminal dopaminergic fibers in the striatum. Knockdown of PINK1 by itself, however, did not affect the number of DN in this 3-week time frame (Table 3) (Haque et al. 2012). Recent observations from our own group have confirmed that knockdown of PINK1 (Oliveras-Salvá et al. 2014) or even parkin using rAAV2/7 in adult mouse or rat SN up to 10 months does not elicit significant dopaminergic cell death (unpublished data).

Table 3 Other viral vector-based rodent PD models

Gene	Vector system	Promoter	Species	Injection site	DN loss in SN	Time course	α-SYN aggregates	Ubiq.	phos. S129	Motor phenotype
LRRK2 WT (Dusonchet et al. 2011)	AdV	Synapsin-1	Rat	STR	None	42 days	No	No	No	–
LRRK2 G2019S					10–20 %	10–42 days				
LRRK2 WT (Lee et al. 2010)	HSV	HSV IE 4/5	Mice	STR	20 %	3 weeks	–	–	–	–
LRRK2 G2019S					50 %					
LRRK2 G2019S/D1994A					20 %					
Parkin Con. KD (Shin et al. 2011)	LV	n.d.	Mice	SN	45 %	10 months	–	–	–	–
PINK KD (Haque et al. 2012)	AdV	CMV	Mice	STR	None	3 weeks	–	–	–	–
PINK1 KD + MPTP					45 %					
CTR + MPTP					17 %					

AdV adenoviral vector; *HSV* herpes simplex vector; *LV* lentiviral vector; *HSV IE4/5* HSV immediate-early 4/5 gene promoter; *Ubiq* ubiquitination; *posph.S129* S129 α-synuclein phosphorylation; – not determined; *n.d.* not described; *SN* substantia nigra; *STR* striatum

However, an elegant alternative approach was recently pursued by adult knockout of parkin after injection of an LV expressing GFP-Cre into the SN of 6- to 8-week-old conditional parkin knockout mice (exon 7 flanked by loxP sites, parkin$^{FLX/FLX}$). Injection of LV GFP-Cre led to a near complete loss of parkin in the ventral midbrain of parkin$^{Flx/Flx}$ animals, resulting in a significant reduction in nigral DN up to 45 % after 10 months (Shin et al. 2011).

5 Conclusions and Perspectives

Much effort has been spent over the last 10 years on the development and improvement of viral vector-based PD models. So far, models based on overexpression of α-synuclein are the most prevalent and extensively studied. The first-generation α-synuclein models have learned that the levels of α-synuclein expression crucially determine the disease onset and severity, and are important to elicit reliable motor impairments. Since then, efforts have been focused on increasing expression levels of α-synuclein in the DN, which has resulted in second- and third-generation models. rAAV vectors have gradually outcompeted LV vectors because of their higher titers and transduction efficiency of DN. The best models currently available have been developed from a combined approach using newer AAV serotypes (rAAV1, 5, 6, 7, 8) and optimized vector constructs, titer, and purity. These third-generation α-synuclein models show improved face and predictive validity and therefore offer the possibility to reliably test novel therapeutics.

Besides being useful for preclinical drug testing, the viral vector-based α-synuclein models have allowed to examine important aspects of α-synuclein pathophysiology, such as α-synuclein phosphorylation, toxicity of α-synuclein oligomers versus fibrils, etc. Furthermore, differences in sensitivity of DN among animal species have been detected. Compared to rats, mice DN seem to be less sensitive to α-synuclein overexpression, resulting in a delayed manifestation of neurodegeneration. This is in line with observations in α-synuclein transgenic mice where dopaminergic degeneration is largely absent. However, a comparison with α-synuclein transgenic rats would be required to rule out whether this is genuinely due to different protective mechanisms between mice and rats rather than to differences in levels or age-of-onset of the α-synuclein overexpression.

Comparing models created across different laboratories remains difficult since different vector systems, serotypes, and production methods are being used. The vector titer as well as the vector purity directly influences the phenotypic outcome of the model. Excessive vector titers or insufficiently purified vector batches may result in a specific toxicity. Therefore, appropriate control vectors are indispensable. Considerable time investment in the viral vector production, upscaling, and purification procedures has also proven essential to obtain reproducible and high quality vector batches.

Rodent models based on overexpression of G2019S LRRK2 display mild (10 %) to severe (50 %) neurodegeneration depending on the vector system (AdV5 or HSV) used. A disadvantage of the AdV5-based rat model is the low percentage of retrogradely transduced nigral neurons (31 %) compared to the 75 % obtained in mice using HSV. Importantly, both models have provided in vivo evidence for the link between G2019S LRRK2 toxicity and elevated kinase activity, which reinforces the concept of LRRK2 kinase inhibition as a possible therapeutic strategy.

RNAi-mediated viral vector-based knockdown offers in principle an interesting tool to study recessive genes like parkin, DJ-1, and PINK1. However, until now, only one study related to PINK1 has been published applying this approach. This might partially be explained by difficulties to detect and quantify knockdown levels in vivo due to the lack of reliable antibodies or other detection methods of endogenous protein. Nevertheless, the knockdown approach with viral vectors still represents an attractive and flexible strategy to combine with overexpression/knockdown of other PD-related genes or also in combination with transgenic animals in order to determine whether these proteins act in similar pathways in vivo.

References

Andres-Mateos E, Perier C, Zhang L, Blanchard-Fillion B, Greco TM, Thomas B, Ko HS, Sasaki M, Ischiropoulos H, Przedborski S, Dawson TM, Dawson VL (2007) DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc Natl Acad Sci U S A 104(37):14807–14812

Atchison RW, Casto BC, Hammon WM (1965) Adenovirus-associated defective virus particles. Science 149:754–756

Azeredo da Silveira S, Schneider BL, Cifuentes-Diaz C, Sage D, Abbas-Terki T, Iwatsubo T, Unser M, Aebischer P (2009) Phosphorylation does not prompt, nor prevent, the formation of alpha-synuclein toxic species in a rat model of Parkinson's disease. Hum Mol Genet 18(5):872–887

Baekelandt V, De Strooper B, Nuttin B, Debyser Z (2000) Gene therapeutic strategies for neurodegenerative diseases. Curr Opin Mol Ther 2(5):540–554

Baekelandt V, Claeys A, Eggermont K, Lauwers E, De Strooper B, Nuttin B, Debyser Z (2002) Characterization of lentiviral vector-mediated gene transfer in adult mouse brain. Hum Gene Ther 13(7):841–853

Bankiewicz KS, Eberling JL, Kohutnicka M, Jagust W, Pivirotto P, Bringas J, Cunningham J, Budinger TF, Harvey-White J (2000) Convection-enhanced delivery of AAV vector in parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach. Exp Neurol 164(1):2–14

Belin AC, Westerlund M (2008) Parkinson's disease: a genetic perspective. FEBS J 275(7):1377–1383

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3(12):1301–1306

Betarbet R, Sherer TB, Greenamyre JT (2002) Animal models of Parkinson's disease. BioEssays 24(4):308–318

Bezard E, Przedborski S (2011) A tale on animal models of Parkinson's disease. Mov Disord 26(6):993–1002

Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. Proc Natl Acad Sci U S A 99(4):2344–2349

Blouin V, Brument N, Toublanc E, Raimbaud I, Moullier P, Salvetti A (2004) Improving rAAV production and purification: towards the definition of a scaleable process. J Gene Med 6(Suppl 1):S223–S228

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24(2):197–211

Bukovsky AA, Song JP, Naldini L (1999) Interaction of human immunodeficiency virus-derived vectors with wild-type virus in transduced cells. J Virol 73(8):7087–7092

Buller RM, Janik JE, Sebring ED, Rose JA (1981) Herpes simplex virus types 1 and 2 completely help adenovirus-associated virus replication. J Virol 40(1):241–247

Burger C, Gorbatyuk OS, Velardo MJ, Peden CS, Williams P, Zolotukhin S, Reier PJ, Mandel RJ, Muzyczka N (2004) Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol Ther 10(2):302–317

Burger C, Nguyen FN, Deng J, Mandel RJ (2005) Systemic mannitol-induced hyperosmolality amplifies rAAV2-mediated striatal transduction to a greater extent than local co-infusion. Mol Ther 11(2):327–331

Burton EA, Fink DJ, Glorioso JC (2002) Gene delivery using herpes simplex virus vectors. DNA Cell Biol 21(12):915–936

Campos SK, Barry MA (2007) Current advances and future challenges in adenoviral vector biology and targeting. Curr Gene Ther 7 (3):189-204

Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M, Waucquier N, Defebvre L, Amouyel P, Farrer M, Destee A (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364(9440):1167–1169

Chen L, Thiruchelvam MJ, Madura K, Richfield EK (2006) Proteasome dysfunction in aged human alpha-synuclein transgenic mice. Neurobiol Dis 23(1):120–126

Chesselet MF, Richter F (2011) Modelling of Parkinson's disease in mice. Lancet Neurol 10(12):1108–1118

Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, VanBrocklin HF, Wright JF, Bankiewicz KS, Aminoff MJ (2009) Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. Neurology 73(20):1662–1669

Chu D, Thistlethwaite PA, Sullivan CC, Grifman MS, Weitzman MD (2004) Gene delivery to the mammalian heart using AAV vectors. Methods Mol Biol 246:213–224

Chung CY, Koprich JB, Siddiqi H, Isacson O (2009) Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. J Neurosci 29(11):3365–3373

Civiero L, Vancraenenbroeck R, Belluzzi E, Beilina A, Lobbestael E, Reyniers L, Gao F, Micetic I, De Maeyer M, Bubacco L, Baekelandt V, Cookson MR, Greggio E, Taymans JM (2012) Biochemical characterization of highly purified leucine-rich repeat kinases 1 and 2 demonstrates formation of homodimers. PLoS One 7(8)

Clark KR, Voulgaropoulou F, Fraley DM, Johnson PR (1995) Cell lines for the production of recombinant adeno-associated virus. Hum Gene Ther 6(10):1329–1341

Cunningham J, Oiwa Y, Nagy D, Podsakoff G, Colosi P, Bankiewicz KS (2000) Distribution of AAV-TK following intracranial convection-enhanced delivery into rats. Cell Transplant 9(5):585–594

Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. Neuron 39(6):889–909

Davison AJ, Benko M, Harrach B (2003) Genetic content and evolution of adenoviruses. J Gen Virol 84(Pt 11):2895–2908

De Rijck J, Van Maele B, Debyser Z (2005) Positional effects of the central DNA flap in HIV-1-derived lentiviral vectors. Biochem Biophys Res Commun 328(4):987–994

de Silva S, Bowers WJ (2009) Herpes virus amplicon vectors. Viruses 1(3):594–629

Decressac M, Mattsson B, Lundblad M, Weikop P, Bjorklund A (2012) Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of alpha-synuclein in midbrain dopamine neurons. Neurobiol Dis 45(3):939–953

Deroose CM, Reumers V, Debyser Z, Baekelandt V (2009) Seeing genes at work in the living brain with non-invasive molecular imaging. Curr Gene Ther 9(3):212–238

Dodiya HB, Bjorklund T, Stansell Iii J, Mandel RJ, Kirik D, Kordower JH (2009) Differential transduction following basal ganglia administration of distinct pseudotyped AAV capsid serotypes in nonhuman primates. Mol Ther 18(3):579–587

Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L (1998) A third-generation lentivirus vector with a conditional packaging system. J Virol 72(11):8463–8471

Dusonchet J, Kochubey O, Stafa K, Young SM Jr, Zufferey R, Moore DJ, Schneider BL, Aebischer P (2011) A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. J Neurosci 31(3):907–912

Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, Wang L, Zuchner S, Konidari I, Wang G, Singer C, Nahab F, Scott B, Stajich JM, Pericak-Vance M, Haines J, Vance JM, Martin ER (2010) Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. Ann Hum Genet 74(2):97–109

Eslamboli A, Romero-Ramos M, Burger C, Bjorklund T, Muzyczka N, Mandel RJ, Baker H, Ridley RM, Kirik D (2007) Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 130(Pt 3):799–815

Farrer MJ (2006) Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 7(4):306–318

Fleming SM, Fernagut PO, Chesselet MF (2005) Genetic mouse models of parkinsonism: strengths and limitations. NeuroRx 2(3):495–503

Fox SH, Brotchie JM (2010) The MPTP-lesioned non-human primate models of Parkinson's disease. Past, present, and future. Prog Brain Res 184:133–157

Freichel C, Neumann M, Ballard T, Muller V, Woolley M, Ozmen L, Borroni E, Kretzschmar HA, Haass C, Spooren W, Kahle PJ (2007) Age-dependent cognitive decline and amygdala pathology in alpha-synuclein transgenic mice. Neurobiol Aging 28(9):1421–1435

Gao G, Qu G, Burnham MS, Huang J, Chirmule N, Joshi B, Yu QC, Marsh JA, Conceicao CM, Wilson JM (2000) Purification of recombinant adeno-associated virus vectors by column chromatography and its performance in vivo. Hum Gene Ther 11(15):2079–2091

Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM (2002) Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proc Natl Acad Sci U S A 99(18):11854–11859

Gao G, Alvira MR, Somanathan S, Lu Y, Vandenberghe LH, Rux JJ, Calcedo R, Sanmiguel J, Abbas Z, Wilson JM (2003) Adeno-associated viruses undergo substantial evolution in primates during natural infections. Proc Natl Acad Sci U S A 100(10):6081–6086

Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM (2004) Clades of Adeno-associated viruses are widely disseminated in human tissues. J Virol 78(12):6381–6388

Gao G, Vandenberghe LH, Wilson JM (2005) New recombinant serotypes of AAV vectors. Curr Gene Ther 5(3):285–297

Gautier CA, Kitada T, Shen J (2008) Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. Proc Natl Acad Sci U S A 105(32):11364–11369

Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ, Gajendiran M, Roth BL, Chesselet MF, Maidment NT, Levine MS, Shen J (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278(44):43628–43635

Goldberg MS, Pisani A, Haburcak M, Vortherms TA, Kitada T, Costa C, Tong Y, Martella G, Tscherter A, Martins A, Bernardi G, Roth BL, Pothos EN, Calabresi P, Shen J (2005) Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45(4):489–496

Gorbatyuk OS, Li S, Sullivan LF, Chen W, Kondrikova G, Manfredsson FP, Mandel RJ, Muzyczka N (2008) The phosphorylation state of Ser-129 in human alpha-synuclein determines neurodegeneration in a rat model of Parkinson disease. Proc Natl Acad Sci U S A 105(2):763–768

Graham FL, Prevec L (1995) Methods for construction of adenovirus vectors. Mol Biotechnol 3 (3):207–220

Grieger JC, Samulski RJ (2005) Packaging capacity of adeno-associated virus serotypes: impact of larger genomes on infectivity and postentry steps. J Virol 79(15):9933–9944

Grimm D, Kay MA (2003) From virus evolution to vector revolution: use of naturally occurring serotypes of adeno-associated virus (AAV) as novel vectors for human gene therapy. Curr Gene Ther 3(4):281–304

Hadaczek P, Mirek H, Bringas J, Cunningham J, Bankiewicz K (2004) Basic fibroblast growth factor enhances transduction, distribution, and axonal transport of adeno-associated virus type 2 vector in rat brain. Hum Gene Ther 15(5):469–479

Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, Kay DM, Doheny KF, Paschall J, Pugh E, Kusel VI, Collura R, Roberts J, Griffith A, Samii A, Scott WK, Nutt J, Factor SA, Payami H (2010) Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nat Genet 42(9):781–785

Haque ME, Mount MP, Safarpour F, Abdel-Messih E, Callaghan S, Mazerolle C, Kitada T, Slack RS, Wallace V, Shen J, Anisman H, Park DS (2012) Inactivation of Pink1 gene in vivo sensitizes dopamine-producing neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and can be rescued by autosomal recessive Parkinson disease genes, Parkin or DJ-1. J Biol Chem 287(27):23162–23170

Hawkes CH, Del Tredici K, Braak H (2010) A timeline for Parkinson's disease. Parkinsonism Relat Disord 16(2):79–84

Hellstrom M, Ruitenberg MJ, Pollett MA, Ehlert EM, Twisk J, Verhaagen J, Harvey AR (2009) Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection. Gene Ther 16(4):521–532

Hoglinger GU, Oertel WH, Hirsch EC (2006) The rotenone model of parkinsonism—the five years inspection. J Neural Transm Suppl 70:269–272

Howarth JL, Lee YB, Uney JB (2010) Using viral vectors as gene transfer tools (Cell Biology and Toxicology Special Issue: ETCS-UK 1 day meeting on genetic manipulation of cells). Cell Biol Toxicol 26(1):1–20

Ibanez P, Bonnet AM, Debarges B, Lohmann E, Tison F, Pollak P, Agid Y, Durr A, Brice A (2004) Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet 364(9440):1169–1171

Ilijic E, Guzman JN, Surmeier DJ (2011) The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. Neurobiol Dis 43(2):364–371

Itier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA, Laville M, Pratt J, Corti O, Pradier L, Ret G, Joubert C, Periquet M, Araujo F, Negroni J, Casarejos MJ, Canals S, Solano R, Serrano A, Gallego E, Sanchez M, Denefle P, Benavides J, Tremp G, Rooney TA, Brice A, Garcia de Yebenes J (2003) Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet 12(18):2277–2291

Jiang H, Jackson-Lewis V, Muthane U, Dollison A, Ferreira M, Espinosa A, Parsons B, Przedborski S (1993) Adenosine receptor antagonists potentiate dopamine receptor agonist-induced rotational behavior in 6-hydroxydopamine-lesioned rats. Brain Res 613(2):347–351

Kahle PJ, Neumann M, Ozmen L, Muller V, Odoy S, Okamoto N, Jacobsen H, Iwatsubo T, Trojanowski JQ, Takahashi H, Wakabayashi K, Bogdanovic N, Riederer P, Kretzschmar HA, Haass C (2001) Selective insolubility of alpha-synuclein in human Lewy body diseases is recapitulated in a transgenic mouse model. Am J Pathol 159(6):2215–2225

Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, Bland RJ, Young D, Strybing K, Eidelberg D, During MJ (2007) Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. Lancet 369(9579):2097–2105

Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, Wakeham A, You-Ten AJ, Kalia SK, Horne P, Westaway D, Lozano AM, Anisman H, Park DS, Mak TW (2005) Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc Natl Acad Sci U S A 102(14):5215–5220

Kirik D, Georgievska B, Burger C, Winkler C, Muzyczka N, Mandel RJ, Bjorklund A (2002a) Reversal of motor impairments in parkinsonian rats by continuous intrastriatal delivery of L-dopa using rAAV-mediated gene transfer. Proc Natl Acad Sci U S A 99(7):4708–4713

Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ, Bjorklund A (2002b) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J Neurosci 22(7):2780–2791

Kirik D, Annett LE, Burger C, Muzyczka N, Mandel RJ, Bjorklund A (2003) Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease. Proc Natl Acad Sci U S A 100(5):2884–2889

Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C, Pothos EN, Shen J (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc Natl Acad Sci U S A 104(27):11441–11446

Klein RL, King MA, Hamby ME, Meyer EM (2002) Dopaminergic cell loss induced by human A30P alpha-synuclein gene transfer to the rat substantia nigra. Hum Gene Ther 13(5):605–612

Koprich JB, Johnston TH, Huot P, Reyes MG, Espinosa M, Brotchie JM (2011) Progressive neurodegeneration or endogenous compensation in an animal model of Parkinson's disease produced by decreasing doses of alpha-synuclein. PLoS ONE 6(3):e17698

Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18(2):106–108

Lauwers E, Debyser Z, Van Dorpe J, De Strooper B, Nuttin B, Baekelandt V (2003) Neuropathology and neurodegeneration in rodent brain induced by lentiviral vector-mediated overexpression of alpha-synuclein. Brain Pathol 13(3):364–372

Lauwers E, Beque D, Van Laere K, Nuyts J, Bormans G, Mortelmans L, Casteels C, Vercammen L, Bockstael O, Nuttin B, Debyser Z, Baekelandt V (2007) Non-invasive imaging of neuropathology in a rat model of alpha-synuclein overexpression. Neurobiol Aging 28(2):248–257

Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, Lee YI, Maguire-Zeiss KA, Bowers WJ, Federoff HJ, Dawson VL, Dawson TM (2010) Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. Nat Med 16(9):998–1000

Lesage S, Brice A (2009) Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum Mol Genet 18(R1):R48–R59

Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C (2009) Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci 12(7):826–828

Li X, Patel JC, Wang J, Avshalumov MV, Nicholson C, Buxbaum JD, Elder GA, Rice ME, Yue Z (2010) Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. J Neurosci 30(5):1788–1797

Lo Bianco C, Ridet JL, Schneider BL, Deglon N, Aebischer P (2002) alpha-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci U S A 99(16):10813–10818

Lock M, Alvira M, Vandenberghe LH, Samanta A, Toelen J, Debyser Z, Wilson JM (2010) Rapid, simple and versatile manufacturing of recombinant adeno-associated virus vectors at scale. Hum Gene Ther 21(10):1259–1271

Lu XH, Fleming SM, Meurers B, Ackerson LC, Mortazavi F, Lo V, Hernandez D, Sulzer D, Jackson GR, Maidment NT, Chesselet MF, Yang XW (2009) Bacterial artificial chromosome

transgenic mice expressing a truncated mutant parkin exhibit age-dependent hypokinetic motor deficits, dopaminergic neuron degeneration, and accumulation of proteinase K-resistant alpha-synuclein. J Neurosci 29(7):1962–1976

Ma SY, Roytta M, Rinne JO, Collan Y, Rinne UK (1997) Correlation between neuromorphometry in the substantia nigra and clinical features in Parkinson's disease using dissector counts. J Neurol Sci 151(1):83–87

Magen I, Chesselet MF (2010) Genetic mouse models of Parkinson's disease—the state of the art. Prog Brain Res 184:53–87

Maingay M, Romero-Ramos M, Carta M, Kirik D (2006) Ventral tegmental area dopamine neurons are resistant to human mutant alpha-synuclein overexpression. Neurobiol Dis 23(3):522–532

Mandel RJ (2010) CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer's disease. Curr Opin Mol Ther 12(2):240–247

Manfredsson FP, Burger C, Sullivan LF, Muzyczka N, Lewin AS, Mandel RJ (2007) rAAV-mediated nigral human parkin over-expression partially ameliorates motor deficits via enhanced dopamine neurotransmission in a rat model of Parkinson's disease. Exp Neurol 207(2):289–301

Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, Palmer CA, Feigenbaum F, Tornatore C, Tufaro F, Martuza RL (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 7(10):867–874

Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287(5456):1265–1269

Mastakov MY, Baer K, Xu R, Fitzsimons H, During MJ (2001) Combined injection of rAAV with mannitol enhances gene expression in the rat brain. Mol Ther 3(2):225–232

Mastakov MY, Baer K, Kotin RM, During MJ (2002) Recombinant adeno-associated virus serotypes 2- and 5-mediated gene transfer in the mammalian brain: quantitative analysis of heparin co-infusion. Mol Ther 5(4):371–380

McFarland NR, Lee JS, Hyman BT, McLean PJ (2009a) Comparison of transduction efficiency of recombinant AAV serotypes 1, 2, 5, and 8 in the rat nigrostriatal system. J Neurochem 109(3):838–845

McFarland NR, Fan Z, Xu K, Schwarzschild MA, Feany MB, Hyman BT, McLean PJ (2009b) Alpha-synuclein S129 phosphorylation mutants do not alter nigrostriatal toxicity in a rat model of Parkinson disease. J Neuropathol Exp Neurol 68(5):515–524

Miao CH, Snyder RO, Schowalter DB, Patijn GA, Donahue B, Winther B, Kay MA (1998) The kinetics of rAAV integration in the liver. Nat Genet 19(1):13–15

Mochizuki H, Yamada M, Mizuno Y (2006) Alpha-synuclein overexpression model. J Neural Transm Suppl 70:281–284

Moskalenko M, Chen L, van Roey M, Donahue BA, Snyder RO, McArthur JG, Patel SD (2000) Epitope mapping of human anti-adeno-associated virus type 2 neutralizing antibodies: implications for gene therapy and virus structure. J Virol 74(4):1761–1766

Naldini L, Blomer U, Gage FH, Trono D, Verma IM (1996a) Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proc Natl Acad Sci U S A 93(21):11382–11388

Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D (1996b) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272(5259):263–267

Oliveras-Salvá M, Macchi F, Coessens V, Deleersnijder A, Gérard M, Van der Perren A, Van den Haute C, Baekelandt V (2014) Total but not partial loss of PINK1 enhances α-synuclein-induced neurodegeneration in mouse substantia nigra. Neurobiol Aging

Oliveras-Salvá M, Van der Perren A, Casadei N, Nuber S, D'Hooge R, Van den Haute C, Baekelandt V (2013) rAAV vector-mediated overexpression of α-synuclein in mouse

substantia nigra induces protein aggregation and progressive dose-dependent neurodegeneration. Mol Neurodegener 8:44

Paisan-Ruiz C (2009) LRRK2 gene variation and its contribution to Parkinson disease. Hum Mutat 30(8):1153–1160

Pankratz N, Wilk JB, Latourelle JC, DeStefano AL, Halter C, Pugh EW, Doheny KF, Gusella JF, Nichols WC, Foroud T, Myers RH (2009) Genomewide association study for susceptibility genes contributing to familial Parkinson disease. Hum Genet 124(6):593–605

Passini MA, Watson DJ, Wolfe JH (2004) Gene delivery to the mouse brain with adeno-associated virus. Methods Mol Biol 246:225–236

Paterna JC, Feldon J, Bueler H (2004) Transduction profiles of recombinant adeno-associated virus vectors derived from serotypes 2 and 5 in the nigrostriatal system of rats. J Virol 78(13):6808–6817

Perez FA, Palmiter RD (2005) Parkin-deficient mice are not a robust model of parkinsonism. Proc Natl Acad Sci U S A 102(6):2174–2179

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276(5321):2045–2047

Rabinowitz JE, Samulski RJ (2000) Building a better vector: the manipulation of AAV virions. Virology 278(2):301–308

Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, Dawson TM, Dawson VL, Moore DJ (2011) Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. PLoS ONE 6(4):e18568

Richfield EK, Thiruchelvam MJ, Cory-Slechta DA, Wuertzer C, Gainetdinov RR, Caron MG, Di Monte DA, Federoff HJ (2002) Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. Exp Neurol 175(1):35–48

Rutledge EA, Halbert CL, Russell DW (1998) Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. J Virol 72(1):309–319

Samulski RJ, Berns KI, Tan M, Muzyczka N (1982) Cloning of adeno-associated virus into pBR322: rescue of intact virus from the recombinant plasmid in human cells. Proc Natl Acad Sci U S A 79(6):2077–2081

Samulski RJ, Chang LS, Shenk T (1987) A recombinant plasmid from which an infectious adeno-associated virus genome can be excised in vitro and its use to study viral replication. J Virol 61(10):3096–3101

Sanchez-Guajardo V, Febbraro F, Kirik D, Romero-Ramos M (2010) Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. PLoS ONE 5(1):e8784

Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 41(12):1303–1307

Schmidt M, Grot E, Cervenka P, Wainer S, Buck C, Chiorini JA (2006) Identification and characterization of novel adeno-associated virus isolates in ATCC virus stocks. J Virol 80(10):5082–5085

Shelkovnikova TA, Ustyugov AA, Millership S, Peters O, Anichtchik O, Spillantini MG, Buchman VL, Bachurin SO, Ninkina NN (2011) Dimebon does not ameliorate pathological changes caused by expression of truncated (1-120) human alpha-synuclein in dopaminergic neurons of transgenic mice. Neurodegener Dis 8(6):430–437

Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL, Dawson TM (2011) PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. Cell 144(5):689–702

Shults CW (2006) Lewy bodies. Proc Natl Acad Sci U S A 103(6):1661–1668

Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41(12):1308–1312

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) alpha-Synuclein locus triplication causes Parkinson's disease. Science 302(5646):841

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388(6645):839–840

St Martin JL, Klucken J, Outeiro TF, Nguyen P, Keller-McGandy C, Cantuti-Castelvetri I, Grammatopoulos TN, Standaert DG, Hyman BT, McLean PJ (2007) Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. J Neurochem 100(6):1449–1457

Taymans JM, Vandenberghe LH, Haute CV, Thiry I, Deroose CM, Mortelmans L, Wilson JM, Debyser Z, Baekelandt V (2007) Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain. Hum Gene Ther 18(3):195–206

Tenenbaum L, Chtarto A, Lehtonen E, Velu T, Brotchi J, Levivier M (2004) Recombinant AAV-mediated gene delivery to the central nervous system. J Gene Med 6(Suppl 1):S212–S222

Theodore S, Cao S, McLean PJ, Standaert DG (2008) Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J Neuropathol Exp Neurol 67(12):1149–1158

Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA (2000) Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? Brain Res 873(2):225–234

Thiruchelvam MJ, Powers JM, Cory-Slechta DA, Richfield EK (2004) Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. Eur J Neurosci 19(4):845–854

Toelen J, Van der Perren A, Carlon M, Michiels M, Lock M, Vandenberghe L, Bannert N, Wilson JM, Gijsbers R, Debyser Z (2014) Novel approach to research grade AAV vector manufacturing and separation of distinct AAV serotypes for in vivo applications (in revision)

Tofaris GK, Garcia Reitbock P, Humby T, Lambourne SL, O'Connell M, Ghetti B, Gossage H, Emson PC, Wilkinson LS, Goedert M, Spillantini MG (2006) Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J Neurosci 26 (15):3942–3950

Tong Y, Pisani A, Martella G, Karouani M, Yamaguchi H, Pothos EN, Shen J (2009) R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice. Proc Natl Acad Sci U S A 106(34):14622–14627

Ulusoy A, Sahin G, Bjorklund T, Aebischer P, Kirik D (2009) Dose optimization for long-term rAAV-mediated RNA interference in the nigrostriatal projection neurons. Mol Ther 17(9): 1574–1584

Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res 24(3):485–493

Van der Perren A, Toelen J, Carlon M, Van den Haute C, Coun F, Heeman B, Reumers V, Vandenberghe LH, Wilson JM, Debyser Z, Baekelandt V (2011) Efficient and stable

transduction of dopaminergic neurons in rat substantia nigra by rAAV 2/1, 2/2, 2/5, 2/6.2, 2/7, 2/8 and 2/9. Gene Ther 18(5):517–527

Van der Perren A, Macchi F, Toelen J, Van Rompuy AS, Casteels C, Sarre S, Gerard G, Casadei N, Nuber S, Himmelreich U, Osorio Garcia MI, Michotte Y, Bormans G, Van Laere K, Gijsbers R, Van den Haute C, Debyser Z, Baekelandt V (2014) Longitudinal characterization of a robust rat model for Parkinson's disease based on overexpression of alpha-synuclein with rAAV2/7 viral vectors (in revision)

Vercammen L, Van der Perren A, Vaudano E, Gijsbers R, Debyser Z, Van den Haute C, Baekelandt V (2006) Parkin protects against neurotoxicity in the 6-hydroxydopamine rat model for Parkinson's disease. Mol Ther 14(5):716–723

Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM (2004) Loss of locus coeruleus neurons and reduced startle in parkin null mice. Proc Natl Acad Sci U S A 101(29):10744–10749

Wakamatsu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, Kanbe D, Muramatsu S, Kobayashi K, Iwatsubo T, Yoshimoto M (2008) Selective loss of nigral dopamine neurons induced by overexpression of truncated human alpha-synuclein in mice. Neurobiol Aging 29(4):574–585

Wang L, Qi X, Shen R, Sun Y, Tuveson DA (2009) An shRNA silencing a non-toxic transgene reduces nutrient consumption and increases production of adenoviral vectors in a novel packaging cell. J Cell Physiol 219(2):365–371

Winklhofer KF (2007) The parkin protein as a therapeutic target in Parkinson's disease. Expert Opin Ther Targets 11(12):1543–1552

Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S, Tzitzilonis C, Soragni A, Jessberger S, Mira H, Consiglio A, Pham E, Masliah E, Gage FH, Riek R (2011) In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A 108(10):4194–4199

Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 26(Suppl 1):S1–S58

Wright JF (2009) Transient transfection methods for clinical adeno-associated viral vector production. Hum Gene Ther 20(7):698–706

Wu Z, Asokan A, Samulski RJ (2006) Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol Ther 14(3):316–327

Xiao X, Li J, Samulski RJ (1996) Efficient long-term gene transfer into muscle tissue of immunocompetent mice by adeno-associated virus vector. J Virol 70(11):8098–8108

Yamada M, Iwatsubo T, Mizuno Y, Mochizuki H (2004) Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. J Neurochem 91(2):451–461

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Llorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yebenes JG (2004) The new mutation, E46 K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55(2):164–173

Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P (2000) HIV-1 genome nuclear import is mediated by a central DNA flap. Cell 101(2):173–185

Zhu XR, Maskri L, Herold C, Bader V, Stichel CC, Gunturkun O, Lubbert H (2007) Non-motor behavioural impairments in parkin-deficient mice. Eur J Neurosci 26(7):1902–1911

Zufferey R, Nagy D, Mandel RJ, Naldini L, Trono D (1997) Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat Biotechnol 15(9):871–875

Zufferey R, Donello JE, Trono D, Hope TJ (1999) Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. J Virol 73(4):2886–2892

Transgenic Rodent Models to Study Alpha-Synuclein Pathogenesis, with a Focus on Cognitive Deficits

Asa Hatami and Marie-Francoise Chesselet

Abstract The aggregation of alpha-synuclein (aSyn) has been implicated in a number of degenerative diseases collectively termed synucleinopathies. Although most cases of synucleinopathies are idiopathic in nature, there are familial cases of these diseases that are due to mutations or multiplications of the gene coding for aSyn. Two of the most common synucleinopathies are Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Both of these diseases present with cognitive deficits, though with different clinical and temporal features. In PD, cognitive deficits are subtle, may occur before the onset of the classical motor symptoms, and only occasionally lead to dementia in the later stages of the disease. In contrast, dementia is the dominating feature of DLB from the disease onset. The impact of aSyn pathology on the development of neurobiological and behavioral impairments can be investigated using rodent models. There are currently several lines of transgenic mice overexpressing wild-type or mutated aSyn under various promoters. This review will provide an updated synopsis of the mouse lines available, summarize their cognitive deficits, and reflect on how deficits observed in these mice relate to the disease process in humans. In addition, we will review mouse lines where knockout strategies have been applied to study the effects of aSyn on various cognitive tasks and comment on how these lines have been used in combination with other transgenic strains, or with human aSyn overexpression by viral vectors. Finally, we will discuss the recent advent of bacterial artificial chromosome (BAC) transgenic models of PD and their effectiveness in modeling cognitive decline in PD.

Keywords Parkinson's disease · Dementia · Behavior · Mouse · Rat

A. Hatami · M.-F. Chesselet (✉)
Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, USA
e-mail: mchesselet@mednet.ucla.edu

A. Hatami
e-mail: ahatami@mednet.ucla.edu

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1 Introduction

Alpha-synuclein (aSyn) is a 140-amino acid-long protein that is encoded by the *SNCA* gene and is normally expressed in neurons in the central and peripheral nervous systems. aSyn is the major protein implicated in the pathogenesis of Parkinson's disease (PD), as its aggregates form the primary components of Lewy bodies and Lewy neurites, which are the pathological hallmarks of this disease (Spillantini et al. 1998). In addition, there are three missense mutations in the *SNCA* gene that are implicated in familial forms of PD.

In Parkinson's disease, it is common for patients to display executive and attentional cognitive deficits before the appearance of the classical motor symptoms of the disease (Rodriguez-Oroz et al. 2009; Watson and Leverenz 2010; Pfeiffer et al. 2013; Yarnall et al. 2013). A subset of these patients will then go on to have more severe cognitive symptoms and develop dementia (Aarsland and Kurz 2010; Pagonabarraga and Kulisevsky 2012). In fact, the existence of mild cognitive deficits in early PD has been shown to be a major risk factor for the development of more severe cognitive problems and dementia later in the course of the disease (Janvin et al. 2006; Williams-Gray et al. 2007). Dementia with Lewy bodies (DLB) is a synucleinopathy distinct from PD, which is characterized by the presentation of cognitive deficits associated with widespread appearance of aSyn aggregates in the cerebral cortex early in the course of the disease (Geser et al. 2005). DLB can be clinically distinguished from PD, as it is chiefly characterized by cognitive symptoms, whereas extrapyramidal motor symptoms predominate in PD (McKeith et al. 2005; Morra and Donovick 2013). Although it is still debatable whether DLB and PD are pathologically distinct (Dickson et al. 2009; Halliday et al. 2011), these two diseases are clinically differentiated based on the timeline along which motor and cognitive symptoms appear. Specifically, if cognitive deficits occur within one year of the onset of motor symptoms, the patient is classified as having DLB, while if cognitive and motor symptoms are temporally separated by more than 12 months, the patient is classified as having Parkinson's disease with dementia (PDD) (McKeith et al. 2005; Halliday et al. 2011). Multiple system atrophy (MSA), which

is a synucleinopathy characterized by the aggregation of aSyn in oligodendrocytes rather than neurons, is also associated with cognitive deficits, albeit to a lesser extent than DLB (Kao et al. 2009; Brown et al. 2010).

In a study of a large cohort of patients with various diseases characterized by the presence of Lewy bodies, the density of aSyn pathology was strongly linked to lower scores on the Mini-Mental State Examination (MMSE), which is a measure of cognitive function (Beach et al. 2009). Intriguingly, the A30P, E46K, and A53T missense mutations all lead to cognitive deficits and dementia, in addition to the classic motor symptoms observed in PD (Krüger et al. 2001; Spira et al. 2001; Yamaguchi et al. 2005; Puschmann et al. 2009; Somme et al. 2011). Furthermore, familial PD resulting from multiplications of the *SNCA* gene (Singleton et al. 2003) is often characterized by prominent cognitive symptoms (Farrer et al. 2004), whose age of onset and severity are correlated with the number of *SNCA* gene copies (Ikeuchi et al. 2008). Interestingly, cognitive deficits have also been reported in otherwise healthy siblings of familial PD patients (Kéri et al. 2010). Similarly, polymorphisms in the *SNCA* gene conferring either protection against or susceptibility to PD are reported to have differential effects on cognitive sequence learning in healthy subjects (Kéri et al. 2008).

Together, these data provide compelling evidence linking aSyn to the development of cognitive deficits and dementia. As a result, mouse models of alpha-synuclein expression have become important avenues wherein the relationships between aSyn pathology and cognitive deficits can be examined. There are currently a large number of aSyn transgenic mouse models, which were created to model a range of pathological and behavioral features of PD and other synucleinopathies. These mouse lines mainly differ in the form of aSyn being expressed (wild type vs. mutated) and its promoter-specific expression pattern (Kahle 2008; Chesselet and Richter 2011; Magen and Chesselet 2011). There are also a number of mouse lines with aSyn deficiency, which have been used to help elucidate the roles of this protein in cellular processes in the brain (Abeliovich et al. 2000; Specht and Schoepfer 2004; Kokhan et al. 2012). This review will focus on studies that have used transgenic aSyn mouse models to investigate the pathological effects of this protein on cognition. We will, however, be excluding mouse models of MSA, as there have been no reports of cognitive deficits in these mice (Fernagut and Tison 2012).

2 Mouse Lines Deficient in aSyn Expression

Considering that there is extensive evidence implicating aSyn's involvement in the development of cognitive deficits in PD-related disorders, studies carried out on mouse models lacking aSyn have been informative in elucidating the contribution of this protein to cognitive function and dysfunction. A naturally occurring mouse model lacking the *SNCA* gene is the OlaHsd subpopulation of the C57BL/6J mouse line (C57BL/6JOlaHsd) (Specht and Schoepfer 2004). These mice were reported to

show deficits in fear extinction when compared to the C57BL/6N line (Stiedl et al. 1999). However, it was later determined that the observed deficits in this cognitive task disappeared when the C57BL/6JOlaHsd mice were compared to C57BL/6JCrl (B6Jax), which had normal aSyn expression (Siegmund et al. 2005), suggesting that the fear extinction deficits observed in the aSyn-deficient mice were unrelated to the expression of this protein. Yet, the C57BL/6JOlaHsd mice are reported to have decreased impulsivity when compared to aSyn-expressing C57BL/6J mice, which raises the possibility of a role for aSyn in the development of impulsivity in PD (Peña-Oliver et al. 2011).

A transgenic mouse line with an aSyn knockout (KO) (Abeliovich et al. 2000) was initially reported to be unimpaired in spatial memory learning, as determined in the Morris Water Maze (MWM) task (Chen et al. 2002). A later study found that there are indeed cognitive deficits in this transgenic model but at older ages compared to the first report, as seen in the MWM and passive and active avoidance tests (Kokhan et al. 2012). One possible confounding factor in elucidating the potential contribution of aSyn to cognitive dysfunction is the compensatory role that gamma-synuclein (gSyn) may play in synaptic regulation in the absence of aSyn, resulting in the amelioration of cognitive deficits in aSyn-deficient models. To address this issue, Senior et al. generated a series of 3 transgenic mouse lines lacking aSyn, gSyn, or both aSyn and gSyn. When these mice were tested in the T-maze, neither single-KO line showed significant cognitive deficits, while the double-KO mice were shown to be impaired (Senior et al. 2008). Furthermore, while the double-KO mice were shown to have unchanged baseline levels of dopamine (DA) in the striatum, they had twofold higher rates of electrically evoked DA release in the striatum when compared to either single-KO line (Senior et al. 2008). The lack of changes in baseline DA release is consistent with previous reports of unchanged DA levels in all 3 of these KO mouse models (Robertson et al. 2004). Taken together, these results support the idea that gSyn plays a compensatory role in the reduction of cognitive deficits in aSyn-deficient mice. Thus, while there is persuasive evidence in favor of a relationship between aSyn gain of function and cognitive deficits (see below), it also appears that the normal expression of aSyn is necessary for proper synaptic function and intact cognition. The cognitive phenotypes of the various aSyn-deficient mouse lines discussed above are summarized in Table 1.

3 Mouse Lines Overexpressing aSyn

There are currently several PD mouse models based on the overexpression of human aSyn under the control of various promoters, as described previously (Magen and Chesselet 2010). When these mice were initially generated, they were mainly described in terms of their motor deficits, with little attention paid to potential cognitive deficits they harbor. Here, we will focus on reviewing the cognitive deficits in each of these models, which are generally more severe in

Table 1 mouse lines deficient in aSyn expression

References	Background	Pathology	Cognitive deficits
Chen et al. (2002)	129/Ola back-crossed to C57BL/6	N/A	None in MWM
Peña-Oliver et al. (2011)		N/A	↓ in impulsivity
Kokhan et al. (2012)		N/A	↓ in MWM at old age
			↓ in passive and active avoidance at old age
Siegmund et al. (2005)	B6JOla	None in dentate gyrus	None in fear conditioning
	B6Jax		
	B6N		
Senior et al. (2008)	C57BL/6	↑ dopamine release in striatum	↓ performance in T-maze only in gSyn double KO

gSyn gamma-synuclein, *MWM* Morris water maze, *N/A* not available

mouse lines using mainly cortical overexpression of the transgene and thus modeling DLB-like pathology. Table 2 below summarizes the characteristics of each mouse line discussed in terms of neuropathology and cognitive deficits.

3.1 Mouse Lines with Extensive Cortical/Hippocampal/ Limbic Pathology

There are a number of mouse models of aSyn overexpression which show extensive pathology in the cortical areas of the brain (Rockenstein et al. 2002; Freichel et al. 2007; Zhou et al. 2008; Schell et al. 2009; Lim et al. 2011). Although these mouse lines are largely proposed to recapitulate features of DLB rather than PD, it is important to note that the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and platelet-derived growth factor subunit β (PDGF-β) models display pathology in the substantia nigra in addition to cortical pathology, which may explain their motor deficits (Masliah et al. 2000; Nuber et al. 2008; Amschl et al. 2013).

3.1.1 Wild-Type Human aSyn Expressed under the PDGF Promoter

The first transgenic mouse line overexpressing human aSyn used PDGF-β as a promoter to drive the transgene (Masliah et al. 2000). These mice were pathologically characterized by sometimes ubiquitinated intraneuronal aSyn inclusions in the temporal neocortex, the CA3 region of the hippocampus, the olfactory bulb, and much more rarely, in the substantia nigra (Masliah et al. 2000). Despite the overwhelmingly cortical pathology, decreases in the numbers of tyrosine hydroxylase (TH)-positive terminals and TH levels in the striatum of the highest expressing mice

Table 2 Mouse lines overexpressing aSyn

References	aSyn variant (promoter)	Background	Expression Levels	Pathology	Cognitive deficits
Rockenstein et al. (2002) Fernagut et al. (2007) Fleming et al. (2008) Lam et al. (2011) Magen et al. (2012)	WT (Mu Thy1)	C57BL/6 X DBA2	2–3X WT levels	Inclusions in SN, LC, and other brain areas No TH+ cell death in SN and LC up to 18 months ↓ ACh and ↑ DA in Cx at 6 months; ↓ NE in Cx at 7 months; ↓ TH and DA in STR at 14 months	↓ Reversal learning at 4–5 months ↓ NOR and NPR at 5–6 months ↓ Y-maze at 5–6 and 7–9 months
Kahle et al. (2000) Freichel et al. (2007) Schell et al. (2009) Schell et al. (2012)	A30P (Mu Thy1)	C57BL/6	~2X WT levels	Inclusions in A, B (TS +), Cx, H, SC, SN, STR Hyperphosphorylated aSyn in cytosol in SC Pser129-aSyn in A and Cx ↓ c-Fos and Plk2 induction in A and H	↓ fear conditioning at 16–19 months ↓ MWM probe trial at 12 months
Rothman et al. (2013) Martin et al. (2014) Rothman et al. (2014)	A53T (Mu Thy1)	C57BL/6 and SV129 backcrossed to C57BL/6	N/A	Transient changes in STR DAT and H serotonin levels at 3 months aSyn aggregates in H, B, C, and SC at 6 months, and throughout CNS at 12 months Apoptotic cell loss in SN, Cx, STR, red nucleus, and Th at 12 months Early apoptotic cell loss in C ↑ mPTP components	None reported
Zhou et al. (2008)	Y39C (Mu Thy1)	FVB/N	150 % WT levels	Pser129-aSyn + ubiquitin + inclusions in Cx Cell loss in Cx	↓ MWM at 15–18 months
Rockenstein et al. (2014)	E57K (Mu Thy1)	C57BL/6 X DBA/2F1	20 % to ~ 3X WT levels	Expression in neuropil, neocortex, limbic sys., BG, and SN No fibrillar or cytosolic aSyn Synaptic inclusions ↓ synapses, neurites, synaptic vesicles, and synapsin 1 in Cx Cell loss in Cx and H	↓ context-dependent learning at 3–4 months
Rieker et al. (2011)	Mouse aSyn (Mu Thy1)	C57BL/6	Up to 6X WT levels	aSyn expression in telencephalon, B, H, C, and SC Ubiquitin+ Pser129+ inclusions and axonal loss in B and SC at 6 months Ubiquitin+ Pser129+ inclusions in H Mitochondrial deficits in SC NMJ dysfunction Novel aSyn form in colliculus and B	None reported

(continued)

Table 2 (continued)

References	aSyn variant (promoter)	Background	Expression Levels	Pathology	Cognitive deficits
Masliah et al. (2000) Masliah et al. (2001) Hashimoto et al. (2003) Winner et al. (2004) Price et al. (2010) Masliah et al. (2011)	WT (PDGF-β)	C57BL/6 X DBA2	10–80 % of human loading control	Inclusions in Cx, H, OB, SN (TH+ cells) Ubiquitination and gliosis in B, C, H, M, and Th ↓ DA, TH, and TH + terminals in SN No ChAT cell loss in STR and NBM up to 22 months ↑ mGluR5 in Cx (colocalization with aSyn) at 6 months ↓ Neurogenesis and ↑ apoptosis in DG ↓ Synaptic density in Cx ↑ Autophagy, ↑ aSyn expression in Cx and H Reversed by immunotherapy	No MWM deficits at 6 months ↓ MWM at 9 months (reversed by MPEP, an mGluR5 antagonist) and 12 months (reversed by immunotherapy) ↑ Thigmotaxis at 12 months
Giasson et al. (2002) Clinton et al. (2010) Paumier et al. (2013) Oaks et al. (2013)	A53T (Mu prion)	C57BL/C3H	∼5–30X WT levels	Inclusions in B, C, SC, STR, and Th Age-dependent ↑ in STR aSyn levels Gliosis, altered neuronal morphology, and Wallerian degeneration in SC Degeneration of sciatic nerve Cell loss in SN at 12 months ↑ STR DAT and DA reuptake Synaptic deficits in H	↓ BCM at 6 months and 9 months (when crossed to 3XTg-AD mice) ↓ Anxiety-like Behavior at 12 months ↓ Y maze at 6 months and 12 months ↓ depressive-like behavior at 4 months
Nuber et al. (2008)	WT (CaM–tTA) (tet off)	C57BL/6	Less than 90 % of human control; slight ↑ in Cx; highest expression in Cx, OB, BG	Trend toward ↓ TH+ neurons in SN ↓ DA in OB and ↓ neurogenesis in DG (reversed by cessation of aSyn expression) Cell loss in H at 20 months	↓ MWM at 12 months
Lim et al. (2011)	WT or A53T (CaM-tTA) (tet off)	C57BL/6 X B6C3H	N/A	Only for A53T: Progressive punctate aSyn staining in H, MB, DG, Cg, Cx, OB, S, and STN at 4–8 months Progressive gliosis in MB at 8–20 months Progressive aSyn phosphorylation in H, OB, Cg, Cx, and aSyn ubiquitination in H, OB, MB at 12–20 months ↑ Cytosolic aSyn and cell loss in H and Cx Synaptic deficits (reversed by transgene expression suppression)	Only for A53T: ↓ Contextual fear memory at 8 months (reversed by cessation of gene expression) No cued fear memory deficits

(continued)

Table 2 (continued)

References	aSyn variant (promoter)	Background	Expression Levels	Pathology	Cognitive deficits
Lin et al. (2012)	A53T (PITX3-tTA)(tet off)	C57BL/6J	2–4X WT levels	aSyn expression mostly in SN, VTA, leaky expression in Cx, B, C, OB, STR, H Cell loss in SN, VTA at 1 month, and in retro-rubral field at 6 months ↓ DA release at months 3–4 months, ↓ STR DA at 6 months STR neuritic changes at 1 month Astrocytosis and microgliosis at 20 months aSyn aggregation at 12 months and 18 months Golgi dysfunction at 12 month Lysosomal and autophagy dysfunction at 1 month ↓ SN Nurr1 at 1 month	None reported

The first reference for each model refers to the originator of the line, and the following references are listed in chronological order. The mouse lines reviewed here are listed in chronological order as well (by date of first publication).
A amygdala, *ACh* acetylcholine, *B* brainstem, *BCM* Barnes circular maze, *BG* basal ganglia, *C* cerebellum, *CaM* calmodulin, *ChAT* choline acetyltransferase, *Cx* cortex, *DA* dopamine, *DAT* dopamine transporter, *DG* dentate gyrus, *H* hippocampus, *LC* locus coeruleus, *M* midbrain, *MB* mammillary bodies, *mGluR5* metabotropic glutamate receptor 5, *mPTP* mitochondrial permeability transition pore, *Mu* murine, *MWM* Morris water maze, *NBM* nucleus basalis of Meynert, *NE* norepinephrine, *NMJ* neuromuscular junction, *NOR* novel object recognition, *NPR* novel place recognition, *OB* olfactory bulb, *Pser129-aSyn* aSyn phosphorylated at serine-129, *SC* spinal cord, *SN* substantia nigra, *STR* striatum, *Th* thalamus, *TH* tyrosine hydroxylase, *TS* thioflavine S, *VTA* ventral tegmental area, and *WT* wild type

have been reported in this model (Hashimoto et al. 2003). The same study also found 25–50 % lower levels of striatal DA, along with increased thigmotaxis in this mouse line at 12 months of age (Hashimoto et al. 2003). However, due to the mainly neocortical nature of the observed pathology in these mice, they have been primarily characterized as models for DLB rather than PD (Rockenstein et al. 2002).

These mice did not show any significant neuronal loss in the nucleus basalis of Meynert, which is involved in the acquisition task in the MWM and is implicated in cognitive decline in humans (Masliah et al. 2001). In the same study, the authors found that these mice did not show significant deficits in the MWM at 6 months of age (Masliah et al. 2001). However, when these mice were tested at 9 months of age in a different study, they were shown to have deficits in spatial learning as indicated in the MWM (Price et al. 2010). These deficits may be due to an upregulation in the levels of the metabotropic glutamate receptor 5(mGluR5) in the cerebral cortex, as they disappeared after the administration of 2-Methyl-6-(phenylethynyl)pyridine (MPEP), which is an mGluR5 inhibitor (Price et al. 2010). Alternatively, the MWM deficits may be due to an observed inhibition of neurogenesis in the hippocampus in this mouse line (Winner et al. 2004). The MWM deficits were also observed at

12 months of age in a separate study and were linked to reductions in both presynaptic terminal diameter and postsynaptic densities in the temporal cortex (Masliah et al. 2011). As expected, these mice show markedly increased aSyn immunoreactivity in the neocortex and the hippocampus. Interestingly, this staining pattern is strongly colocalized with lysosomal and autophagosomal markers (Masliah et al. 2011). When these mice were treated with a C-terminal aSyn antibody in a passive immunization study, they were observed to have lower levels of aSyn aggregates, an amelioration of the previously observed synaptic deficits, and improvements in their cognitive symptoms (Masliah et al. 2011). Taken together, these results more strongly support the idea of aSyn as a causative factor in cognitive decline in synucleinopathies and present a potential therapeutic avenue for the treatment of cognitive deficits in these diseases.

3.1.2 A53T Human aSyn Expressed under the Prion Promoter

One of the mutations in aSyn leading to familial cases of PD is the A53T mutation (Spira et al. 2001). When the A53T mutant form of aSyn is expressed in mice under the prion promoter, it leads to enhanced aggregation of aSyn, along with more severe pathology and behavioral deficits (Giasson et al. 2002). These mice were initially reported to exhibit major pathological changes to their spinal cord motor neurons and develop severe motor impairments, eventually leading to paralysis and death (Giasson et al. 2002). These mice are characterized by motor deficits in the open-field and rotarod tests beginning at 2 months of age and by impairment in the wire hang test starting at 6 months of age (Oaks et al. 2013). These animals also showed reduced anxiety-like behavioral at 12 months of age, and a reduced depressive-like phenotype beginning at 4 months of age (Oaks et al. 2013). Furthermore, these mice showed elevated dopamine transporter (DAT) levels and reuptake potential in synaptosomal fractions of the striatum at young ages, an age-dependent increase in striatal aSyn and phospho-tau accumulation, and nigrostriatal neurodegeneration at 12 months of age (Oaks et al. 2013). While these mice were characterized by motor symptoms, they were not cognitively impaired as observed in the Barnes circular maze (BCM), which is a dry version of the MWM (Clinton et al. 2010). Intriguingly, when these mice were crossed to the 3XTg-AD mice modeling Alzheimer's disease (AD) (Oddo et al. 2003) to produce the DLB-AD mouse model as a part of the same study, they showed significantly higher cognitive deficits than either transgenic model in the BCM at 6 months and 9 months of age (Clinton et al. 2010). These results suggest a toxic interaction between the amyloid-β protein (Aβ) and aSyn, which may be responsible for accelerated and more severe cognitive deficits in diseases such as the Lewy body variant of AD, where the aggregation of multiple proteins is implicated in disease progression (Hansen et al. 1990). In contrast to the preceding results reporting an absence of cognitive symptoms, a recent study using 2-, 6-, and 12-month-old mice from this line reported reduced anxiety-like behavioral at 12 months, spatial memory deficits in the Y-maze at 6 months and 12 months, and grooming and nest-building deficits

and a reduced startle response starting at 2 months of age (Paumier et al. 2013). These deficits were accompanied by synaptic deficits in the hippocampus, a progressive aggregation of aSyn, and gross motor symptoms in old age (Paumier et al. 2013).

3.1.3 aSyn Expressed under the CaMKII Promoter

An excellent promoter for use in reproducing the patterns of hippocampal and cortical pathology observed in DLB is the CaMKII promoter, which leads to high expression of the transgene in the forebrain (Nuber et al. 2008). The expression of wild-type aSyn under the CaMKII promoter in a conditional mouse line resulted in deficits in the MWM at 12 months of age. This cognitive phenotype followed reduced neurogenesis in the hippocampus, which was observed at 6 months of age, and preceded hippocampal neurodegeneration at 20 months of age. As this study was performed in a conditional mouse line, the authors used doxycycline (dox) administration to turn off the expression of the transgene, which led to a restoration of neurogenesis in the hippocampus (Nuber et al. 2008). These results suggest a link between aSyn aggregation, loss of neurogenesis in the hippocampus, and cognitive deficits, which would be strengthened by cognitive testing of the mice following the cessation of transgene expression.

The expression of the A53T mutant form of aSyn under the CaMKinase promoter resulted in impairment of contextual fear memory at 8 months of age, which was significantly correlated with the progressive appearance of aSyn pathology in the hippocampus. Both the contextual fear memory deficits and the hippocampal pathology were reversed when aSyn expression was turned off using dox for a period of 3 months, indicating that these phenotypes are directly related to the expression of the transgene (Lim et al. 2011). Furthermore, in this model, the progressive accumulation of phosphorylated and ubiquitinated aSyn was associated with increasing astrogliosis beginning at 8 months of age and becoming more severe at 20 months. The appearance of posttranslationally modified aSyn species observed in this study also heralded neuronal cell loss in the hippocampus and the cortex, which appeared at 20–22 months of age. The fact that the observed cognitive phenotype precedes hippocampal and cortical neuron loss in this mouse model suggests that the memory deficits observed are unrelated to cell loss and most likely result from synaptic dysfunction present before neurodegeneration (Lim et al. 2011).

3.1.4 Mutant aSyn Expressed under the Thy1 Promoter

Various mouse lines that express the transgene under the Thy1 promoter exhibit highly different patterns of expression of the transgene, and location, and level of pathology. Therefore, it is critical to distinguish the various models that use this

promoter. Several of the Thy1 lines that express mutant alpha-synuclein show extensive pathology in cortex and limbic system and will be described here.

A mouse line overexpressing the A30P variant of aSyn under the control of the murine Thy1 promoter was used to model PD-related cognitive deficits at old ages (Kahle et al. 2000). These mice developed cognitive impairments at 12 months of age, as seen in the MWM (Freichel et al. 2007). They were also reported to be significantly impaired in comparison with wild-type control mice in a fear conditioning task at 16–19 months of age (Schell et al. 2009), which was accompanied by progressive increases in staining for phosphorylated aSyn at residue 129 (Pser129-aSyn) in the brainstem, hippocampus, olfactory bulb, amygdala, and hypothalamus which were present in both presymptomatic and older transgenic mice (Schell et al. 2009). Interestingly, Pser129-aSyn immunoreactivity is characteristic of aSyn aggregates found in PD brain (Fujiwara et al. 2002; Neumann et al. 2002). While the staining pattern for Pser129-aSyn was mainly nuclear and somatic in the cortex and the brainstem, the amygdala and the lateral hypothalamus exhibited neuritic and synaptic Pser129-aSyn staining (Schell et al. 2009). Furthermore, a later study found progressively lower levels of the synaptic plasticity marker c-Fos in the amygdala and hippocampus of this mouse (Schell et al. 2012). This observation was paralleled by an impaired induction of the neuronal activity-responsive kinase Polo-like kinase 2 (Plk2) (Schell et al. 2012). Taken together, these observations suggest a possible link between pathology in the fear-conditioning areas in the brain and the deficits in the fear conditioning task.

While the Y39C mutation in the aSyn sequence is not a naturally occurring mutation found in humans, it is reported to increase protein aggregation and thus leads to the formation of a more neurotoxic protein than wild-type (WT) aSyn. When this variant of aSyn was overexpressed in a mouse line using the Thy1 promoter, the authors observed deficits in the MWM, which began at 15–18 months of age and progressively worsened up to 21–24 months of age. The mutant protein was expressed at 150 % higher levels compared to endogenous aSyn and was distributed in the cortex, hippocampus, thalamus, striatum, and substantia nigra. At 24 months of age, the cortical aSyn expression was characterized by Ser129-phosphorylated and ubiquitinated Lewy body-like structures, and was accompanied by neuronal cell death. Therefore, in this mouse model, significant neuropathology appears after cognitive decline, which begins at 15–18 months of age (Zhou et al. 2008).

Another Thy1-driven mouse model of aSyn overexpression using a non-natural mutation uses the oligomer-prone E57K mutation in aSyn (Rockenstein et al. 2014). The aSyn in this mouse model was expressed in the neocortex, limbic system, basal ganglia, thalamus, and the substantia nigra and was found to be in monomeric and oligomeric forms, whereas the Thy1-WTaSyn mouse line also contained fibrillar species of aSyn. Interestingly, in the E57K mice, aSyn staining was mainly restricted to the neuropil, whereas the Thy1-WTaSyn mouse line had both cytosolic and neuropil staining. This staining pattern was accompanied by the accumulation of aSyn in the synapses (but not the cell bodies), synaptic and dendritic loss, and lower levels of synapsin 1 and synaptic vesicles in the frontal cortex.

In addition, there was significant neurodegeneration in the hippocampus and the frontal cortex in the Thy1-E57KaSyn mice, whereas the Thy1-WTaSyn mice only showed cell loss in the hippocampus. In addition, the highest expressing Thy1-E57KaSyn mice showed impairments in context-dependent learning at 3–4 months of age and performed significantly worse than the Thy1-WTaSyn mice, which are already impaired in this task at 8–10 months of age (Rockenstein et al. 2014). This transgenic mouse model suggests a link between oligomeric aSyn accumulation, synaptic dysfunction, and neuronal cell loss.

In conclusion, several mouse models of alpha-synuclein overexpression show late-onset cognitive deficits that occur before cell loss but correlate with extensive alpha-synuclein pathology in cortex and hippocampus. This is, however, not always the case. For example, mice expressing the A53T aSyn variant under the Thy1 promoter have severe deficits in motor activity, which begin at 6 months of age and end in paralysis and death by 12 months (Martin et al. 2014). These mice also show impairment in the rotarod test at 4.5–6.5 months of age (Rothman et al. 2013). Furthermore, the Thy1-A53TaSyn mice are characterized by hyperactivity in the dark phase and sleep disorders. Additionally, these mice were reported to have transient changes in striatal DAT and hippocampal serotonin levels at 3 months of age (Rothman et al. 2013). These mice express A53T aSyn in the cortex, diencephalon, olfactory bulb, striatum, brainstem, cerebellum, and spinal cord, with aSyn aggregates observed in the hippocampus, brainstem, cerebellum, and spinal cord at 6 months of age and throughout the central nervous system (CNS) at 12 months of age (Martin et al. 2014). This model exhibits robust DA cell loss in the substantia nigra and also shows neurodegeneration of cortical gray and white matter, striatal interneurons, the red nucleus, and the motor thalamus at 12 month of age (Martin et al. 2014). In addition, there is significant cerebellar neuron loss at 12 months of age, with apoptosis of these cells beginning at 1 month of age, in contrast to the later induction of apoptosis in other brain regions (Martin et al. 2014). Therefore, neurodegeneration in this model is widespread and not limited to brain regions primarily involved in PD. The authors also observed a link between increases in the levels of the components of the mitochondrial permeability transition pore (mPTP) and disease pathology (Martin et al. 2014). While the Thy1-A53TaSyn mouse line is characterized by robust cell loss in several different brain regions and is characterized by mitochondrial dysfunction, metabolic abnormalities (Rothman et al. 2014), and motor deficits, there is no cognitive phenotype reported for this mouse model.

3.2 Mouse Lines with Primarily Brainstem Cortico/ Subcortical–Limbic Pathology

Several mouse models of aSyn overexpression have been created to mimic the primarily subcortical nature of PD pathology. Because the main pathological feature of PD is the loss of nigrostriatal dopaminergic neurons, several mouse lines

have been generated that express aSyn in catecholaminergic neurons under the control of the TH promoter (Richfield et al. 2002; Thiruchelvam et al. 2004; Tofaris et al. 2006; Wakamatsu et al. 2008a, b). These mice exhibit various degrees of nigrostriatal pathology (see Chesselet and Richter 2011 for review). However, there have thus far been no reports on the cognitive phenotypes of these mouse models.

3.2.1 Mutant aSyn Expressed under the PITX3 Promoter

In an effort to produce a mouse model characterized by aSyn expression in the dopaminergic neurons of the midbrain, the Cai group generated a line of tetracycline transactivator (tTA)-conditional transgenic mice overexpressing the A53T variant of aSyn. These mice had a pattern of aSyn overexpression restricted to TH-positive neurons of the substantia nigra and the ventral tegmental area, with 2–4 times higher expression of aSyn when compared to non-transgenic controls. In addition, it was determined that aSyn levels in the substantia nigra were about twofold higher when compared to the levels of aSyn in the ventral tegmental area, which is what is expected based on endogenous levels of the pituitary homeobox 3 (PITX3) promoter. However, aSyn expression was also observed in the cortex, brainstem, cerebellum, olfactory bulb, spinal cord, striatum, and hippocampus, most likely due to non-specific expression of the transgene. These transgenic mice displayed early motor deficits as characterized by reduced rearing (improved by cessation of gene expression at embryonic stages) and a shortened and unsteady gait at 1 month, along with impaired horizontal movement and rotarod performance beginning at 2 months. In addition, these mice failed to gain weight as fast as their wild-type controls beginning at 2 months of age. This model is also characterized by progressive neuron loss beginning at 1 month of age in the substantia nigra and the ventral tegmental area, and at 12 months of age in the retro-rubral field. These changes are accompanied by reduced DA release at 3–4 months, a loss of DA in the striatum at 6 months, and striatal neuritic changes beginning at 1 month of age. These mice also exhibited astrocytosis and microgliosis at 20 months, somatic and neuritic aSyn aggregation at 12 and 18 months, Golgi apparatus dysfunction beginning at 12 months, lysosomal and autophagy impairments beginning at 1 month, and a proteasome-dependent loss of nuclear receptor-related 1 (Nurr1) in the substantia nigra at 1 month of age (Lin et al. 2012). In conclusion, while this mouse model achieves targeted expression of A53T aSyn in DA cells of the midbrain and is characterized by early motor deficits and cell loss, it has not been reported to display cognitive features resembling those of early PD.

It is now well established that aSyn pathology in PD is not limited to the nigrostriatal pathway but involves many additional structures. Cross-sectional pathological observations in humans have suggested a staged pattern of disease progression, beginning in the olfactory bulb and ventral medulla; progressing to the raphe nucleus and locus coeruleus; then expanding to the substantia nigra, the nucleus basalis of Meynert, and the amygdala; and finally invading the cerebral cortex (Braak et al. 2002, 2003). Importantly, this pattern of progression is in line

with the clinical evolution of cognitive deficits in PD, which begin with executive and attentional symptoms at the early stages of the disease (Rodriguez-Oroz et al. 2009; Watson and Leverenz 2010) and then progress toward dementia at later disease stages, though only in a subgroup of patients (Aarsland and Kurz 2010; Pagonabarraga and Kulisevsky 2012). Indeed, there is compelling evidence that the progression of cognitive decline in PD parallels the progression of pathology along the aforementioned axis (Braak et al. 2006). Therefore, it is of interest to consider models that reproduce similarly widespread aSyn pathology in addition to strong expression in the vulnerable nigrostriatal dopaminergic neurons. These models include transgenics that express the protein under a widely expressed promoter and BAC mice that have the advantage of reproducing the endogenous pattern of expression of aSyn.

3.2.2 Wild-Type Human aSyn Expressed under the Thy1 Promoter

In contrast to the models described earlier that show late-onset cognitive deficits, one mouse line that overexpresses wild-type human aSyn under the control of the Thy1 promoter (Thy1-aSyn; line 61) (Rockenstein et al. 2002) exhibits deficits at an early age (Magen et al. 2012), in a range of cognitive domains affected in early PD (Gurvich et al. 2007; Peterson et al. 2009; Magen and Chesselet 2010). At 4–5 months of age, naïve male Thy1-aSyn mice showed impaired reversal learning in an operant learning task. Specifically, while these mice were able to learn an operant strategy as well as wild-type control littermates, they were deficient in their ability to reverse their responses in the second phase of the test (Magen et al. 2012). This phenotype recapitulates the early reversal learning deficits observed in PD patients (Peterson et al. 2009). Thy1-aSyn mice also showed impairments in the novel object recognition (NOR) and novel place recognition (NPR) tasks at 4–5 months of age, in addition to deficits in the Y maze spatial alternation task at 5–6 and 7–9 months of age (Magen et al. 2012). However, the transgenic mice did not show a deficit in the Y maze at 3–4 months and 11–13 months of age and were not significantly different from wild-type controls in the cognitive aspects of the holeboard test at 3–4 months (Magen et al. 2012). These results are not surprising, as it has been reported that performance in the Y maze is negatively correlated with extracellular dopamine levels (Li et al. 2010). Indeed, dopamine levels in the striatum of the Thy1-aSyn mice are higher compared to the wild-type control starting at 6 months of age, giving way to progressively lower dopamine levels at 14 months of age (Lam et al. 2011). The fact that deficits observed in the Y maze and NOR tasks both involve the cholinergic system (Yang et al. 2009; Botton et al. 2010) supports the idea that there are also impairments in the cholinergic system in the Thy1-aSyn mouse line. In fact, human aSyn is found in cholinergic neurons in the basal nucleus and the medial septum of these mice, potentially causing toxicity and behavioral deficits. In addition, the levels of acetylcholine are significantly

reduced in the cortex (but not hippocampus) of 6-month-old Thy1-aSyn mice (Magen et al. 2012), which is consistent with the degeneration of cholinergic fibers in the cortex of 6-month-old Thy1-A30PaSyn mice (Szegő et al. 2011).

3.2.3 Mouse aSyn Expressed under the Thy1 Promoter

In order to study the contribution of amino acid differences between human and mouse aSyn, Rieker et al. generated 3 lines of mice overexpressing mouse wild-type aSyn (which naturally includes the A53T substitution observed in a mutant human aSyn variant) at different levels under the control of the Thy1 promoter (Rieker et al. 2011). Similar to some of the Thy1–human–aSyn mice described earlier, these mice expressed the transgene in the telencephalon, brainstem, hippocampus, cerebellar nuclei, and the spinal cord. This expression pattern was accompanied by aSyn pathology and axonal degeneration in the spinal cord and the brainstem at 6 months of age, and by ubiquitination and phosphorylation of aSyn at serine-129. Furthermore, these mice were characterized by deficits in the neuromuscular junction, increased levels of non-ubiquitinated Pser-129-aSyn in the hippocampus, and mitochondrial deficits in the spinal cord. Isoelectric focusing studies also revealed a novel aSyn isotype localized to the colliculus and the brainstem. These mice showed motor impairments as seen in the rotarod test during the first 4 weeks, and also after 6–7 months of age. In conclusion, while mice overexpressing mouse aSyn using the Thy1 promoter are characterized by novel patterns of ubiquitination and phosphorylation and demonstrate mild motor impairments, there were no cognitive deficits reported in this model (Rieker et al. 2011).

3.3 Mice Overexpressing aSyn Using BACs

While traditional transgenic mouse models of PD have been quite useful in reproducing various aspects of Parkinson's disease, they have two major drawbacks. First, since the transgene is under the control of a heterologous promoter, its expression may not necessarily reproduce the temporal and spatial expression pattern of the native protein. Second, the use of traditional transgenic models prevents us from investigating the effects of alternately spliced variants of the gene in question, which may be pathologically relevant. As a result, several groups have attempted to generate mouse models of PD using BACs. BACs allow the integration of the gene of interest into the organism's genome and enable the gene expression to be controlled by its endogenous promoter, giving rise to a more native spatial and temporal expression pattern (Yang and Gong 2001).

There are several mouse models of PD using BACs to overexpress wild-type, A30P, E46K, or A53T aSyn (Kuo et al. 2010; Yamakado et al. 2012; Gardai et al. 2013; Hansen et al. 2013; Janezic et al. 2013; Taylor et al. 2014). The Nussbaum

laboratory was the first one to report on aSyn BAC mice, which overexpressed A30P and A53P variants of aSyn (Kuo et al. 2010). While this mouse line showed 1.3-fold to twofold overexpression of human aSyn in the brain, there were no motor or cognitive deficits reported for these mice (Kuo et al. 2010). Another BAC transgenic mouse line expressing human aSyn, which included the 3' flanking region of the gene, led to an increase in the levels of DAT in striatal synaptosomes and serotonin transporter (SERT) in cortical synaptosomes. These changes were accompanied by hyperlocomotion as seen in the open field at 13 months of age and decreased anxiety-like behavior as seen in the elevated plus maze at 24 months of age (Yamakado et al. 2012). Another BAC mouse line overexpressing aSyn fused to a green fluorescent protein (GFP) construct was reported to display impaired motor performance on the rotarod at 12 months, impaired olfaction at 7 months and 12 months in males, increased aSyn aggregation, and dopaminergic system dysfunction, but no cognitive deficits (Hansen et al. 2013). The Wade–Martins group was also successful in generating a BAC transgenic mouse model overexpressing human aSyn on an *SNCA* null background (Janezic et al. 2013). These mice express striatal aSyn at 1.9-fold higher levels when compared to endogenous expression in C57BL/6 mice. The aSyn expression pattern in these mice recapitulates the endogenous expression pattern and is present in TH-reactive neurons in the substantia nigra and the ventral tegmental area. The transgenic mice showed non-amyloid cytoplasmic aSyn staining in substantia nigra neurons at 18 months of age, which was accompanied by a 30 % cell loss and a reduced firing rate of DA neurons in this region. These mice also displayed age-independent enteric system deficits and age-dependent motor coordination impairment as observed in rotarod, multiple static rods, and forepaw stride length measurements at 18 months of age. Interestingly, the changes in motor performance, DA neuron firing rates, and the cell loss were preceded by reduced dopamine release in the dorsal (but not ventral) striatum beginning at 3–4 months of age. These changes were not dependent on overall DA or DAT levels and were related to the overexpression of aSyn above endogenous levels. Furthermore, the reduced rate of dopamine release in these mice was accompanied by a clustering of synaptic vesicles, but was unrelated to soluble NSF attachment protein (SNAP) receptor (SNARE) complex formation (Janezic et al. 2013). A follow-up study from the same group investigated a pair of BAC transgenic mouse lines expressing either A30P or wild-type aSyn (Taylor et al. 2014) on an *SNCA* null background. These mice showed expression of human aSyn in the cortex, hippocampus, striatum, the ventral tegmental area, and the TH-positive neurons of the substantia nigra, albeit at lower levels when compared to the endogenous expression of aSyn. Interestingly, the expression of aSyn in the DA neurons of the substantia nigra restored their susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in contrast to aSyn KO controls, which were resistant to MPTP-induced cell loss. In addition, the A30P, but not the WT aSyn mice, had reduced electrically evoked dopamine (but not norepinephrine) release in the dorsal striatum. These deficits in DA release were not accompanied by TH-positive cell loss or pathological aSyn aggregation, even at old age. Thus, while these BAC mouse lines are impaired in their DA system responses to electrical

Table 3 BAC transgenic mice expressing aSyn

References	aSyn variant	Background	Expression level	Pathology	Cognitive deficits
Kuo et al. (2010)	A30P or A53T	FVB/N	1.3–2X WT levels in brain	Rare dystrophic synapses in H at 12 months and 22 months No major pathology	N/A
Yamakado et al. (2012)	WT	C57BL6/J	2.7X WT levels	↑ DAT in STR and SERT in Cx	N/A
Hansen et al. (2013)	WT (fused with GFP)	C57BL/6	Highest in OB; High in SN, Cx, H	↑ Phosphorylated aSyn accumulation in Cx, STR, M at 15 months No DA cell loss ↓ DA release	N/A
Janezic et al. (2013)	WT	C57BL/6	1.9X WT levels in STR	Expression in DA neurons of SN and VTA SN cytosolic aSyn staining at 18 months 30 % cell loss and ↓ firing rate in SN ↓ DA release in dorsal STR at 3–4 months Clustering of synaptic vesicles	N/A
Taylor et al. (2014)	WT or A30P	C57BL/6	~10–15X less than WT	No cell loss or aSyn pathology Dysregulation in DA but not NE neurotransmission	N/A

Cx cortex, *DA* dopamine/dopaminergic, *DAT* dopamine transporter, *H* hippocampus, *NE* norepinephrine, *SERT* serotonin transporter, *STR* striatum, and *WT* wild type

stimulation, they do not exhibit any detectable behavioral or pathological deficits (Taylor et al. 2014). Table 3 summarizes the pathological and cognitive characteristics of these models

3.4 Cognitive Deficits in Models of aSyn Overexpression Using Viral Vectors

Viral vectors can be used to deliver recombinant proteins to specific brain regions and may thus be used to model human diseases characterized by region-specific patterns of protein aggregation. The pathological and behavioral outcomes depend

on the viral vector constructs and injection coordinates. Potentially, this approach can thus be used to understand the role of different brain regions, and different levels of aSyn overexpression, in the cognitive deficits caused by aSyn pathology.

Overexpression strategies utilizing viral vectors for delivery of WT or mutant aSyn to the brain were initially developed in rats (Kirik et al. 2002; Lo Bianco et al. 2002; Yamada et al. 2004; Maingay et al. 2006; Hall et al. 2013) and have recently been applied to mouse models (Van der Perren et al. In press). Here, we will review the pathological and phenotypic characteristics of rat PD models using viral vector-mediated transgenic approaches (summarized in Table 4). Kirik et al. generated such a model using either wild-type or A53T mutant aSyn delivered to the substantia nigra of Sprague-Dawley rats (Kirik et al. 2002). These rats widely expressed aSyn in the substantia nigra and the reticular formation, and showed variable aSyn expression in the ventral tegmental area. Furthermore, aSyn expression was observed in the terminal fields of these neurons. These animals displayed a progressive development of aSyn inclusions, dystrophic neurites, and TH-reactive neuron loss at 8 weeks post-injection. In addition, TH activity and DA levels were significantly reduced at 3 weeks post-injection in the striatum of recombinant adeno-associated virus (rAAV)-aSyn animals when compared to the rAAV-GFP controls. Similarly, there was a progressive reduction in nigral DA levels beginning at 3 weeks and continuing to 8 weeks after rAAV injection. These changes were accompanied by significantly higher DA turnover in the rAAV-aSyn animals. The authors also observed motor impairments as seen in the apomorphine rotation test and the paw reaching test. However, these deficits were only observed in the animals that showed a greater-than-60 % DA cell loss (which represented a minor proportion of the animals in the study). Interestingly, no differences were observed between animals treated with WT and A53T aSyn rAAVs (Kirik et al. 2002). Similarly, another model using rAAV-mediated expression of A30P mutant aSyn in the substantia nigra of rats led to aSyn accumulation in the substantia nigra and associated cell loss, along with dystrophic neurites in the substantia nigra and the striatum (Klein et al. 2002). However, no motor or cognitive deficits were observed in this model despite the >50 % cell loss of DA neurons in the substantia nigra (Klein et al. 2002), supporting the idea that cognitive deficits are not solely mediated by alterations in the nigrostriatal dopaminergic system. Another viral delivery model of aSyn overexpression was generated by delivering WT, A53T, or A30P aSyn using a lentiviral vector into the substantia nigra of rats. In this model, the overexpression of all 3 forms of human aSyn, but not rat aSyn, led to progressive TH-reactive cell loss in the substantia nigra at 5 months post-injection. Additionally, WT and A30P (but not A53T) aSyn overexpression led to cell loss in the striatum at the same time point. Furthermore, the lenti-A30P animals showed an increase in the number of degenerating neurons in the substantia nigra when compared with lenti-rat-aSyn animals. Interestingly, the cell loss observed in the substantia nigra of these rats was limited to TH-positive neurons and did not extend to non-DA neurons (Lo Bianco et al. 2002). In a study using rAAV delivery of human aSyn under the control of the cytomegalovirus promoter into the substantia nigra of rats, Yamada et al. observed nigral TH-positive cell loss at 13 weeks post-

Table 4 Models of aSyn overexpression using viral vectors

References	aSyn variant (promoter)	Viral vector	Region	Pathology	Cognitive deficits
Kirik et al. (2002)	WT or A53T (CBA)	AAV	SN	aSyn expression in SN, RF, VTA, and respective terminal fields Progressive ↑ inclusions, ↑ dystrophic neurites, ↑ DA cell loss up to 8 weeks pi ↓ DA levels and TH activity in STR at 3 weeks pi Progressive ↓ SN DA levels at 3–8weeks pi ↑ DA turnover	N/A
Klein et al. (2002)	A30P (CBA)	AAV	SN	aSyn accumulation and >50 % cell loss in SN dystrophic neurites in SN and STR	N/A
Lo Bianco et al. (2002)	WT, A53T, A30P (PGK)	Lentivirus	SN	TH+ cell loss in SN (all 3 isotypes) and STR (only WT and A30P) at 5 months pi	N/A
Yamada et al. (2004)	WT (CMV)	AAV	SN	TH+ cell loss in SN at 13 weeks pi ↑ Pser-129-aSYn and caspase-9 activation	N/A
Maingay et al. (2006)	A53T (CBA)	AAV	VTA	Expression in VTA and projection areas, SN largely spared Intracellular inclusions No cell loss, synaptic loss, or DA loss in VTA	N/A
Hall et al. (2013)	WT (synapsin 1)	AAV	VTA and MS/vDBB	aSyn expression in DA and cholinergic neurons of VTA and MS/vDBB and fiber terminals non-aSyn-specific DA and cholinergic cell loss in VTA and MS/vDBB non-aSyn-specific ↓ ACh in H	↓ working memory and reference learning at 8 weeks pi

ACh acetylcholine, *CBA* chicken β-actin, *CMV* cytomegalovirus, *DA* dopamine, *H* hippocampus, *MS/vDBB* septum and vertical limb of the diagonal band of Broca, *PGK* phosphoglycerate kinase, *pi* post-injection, *Pser-129-aSyn* aSyn phosphorylated at serine-129, *RF* reticular formation, *SN* substantia nigra, *STR* striatum, *TH* tyrosine hydroxylase, and *VTA* ventral tegmental area

injection. The observed cell loss in this model was accompanied by phosphorylation of aSyn at ser-129 and an increase in caspase-9 activation (Yamada et al. 2004). In another study, A53T aSyn was delivered to the ventral tegmental area (VTA) of rats using an rAAV vector (Maingay et al. 2006). The injection of the

construct led to efficient expression of aSyn in the VTA and its projection areas, but largely spared the DA neurons of the substantia nigra. A53T aSyn expression in this model also led to the formation of intracellular inclusions. Interestingly, the overexpression of A53T aSyn did not lead to cell loss in the VTA, but caused significant neuronal loss in the substantia nigra when it was injected there. The VTA neurons in the rAAV-A53T animals were also devoid of synaptic terminal loss and had intact DA levels. Finally, the rAAV-A53T animals showed no impairments in their baseline activity levels when compared to controls, but only showed impairments in the apomorphine test and only at 10 weeks after apomorphine treatment. In a follow-up study, Hall et al. overexpressed WT human aSyn in both the VTA and the septum/vertical limb of the diagonal band of Broca (MS/vDBB) using an rAAV construct and a synapsin 1 promoter. These animals showed robust aSyn expression in the cholinergic and dopaminergic neurons of the VTA and MS/vDBB, along with the fiber terminals of these neurons. While these animals showed both DA and cholinergic neuronal cell loss in the VTA and the MS/vDBB, along with lower acetylcholine (ACh) levels in the hippocampus, these changes are not specific to aSyn and are also observed in rAAV-GFP control animals. However, the rAAV-aSyn animals show significant changes in the apomorphine open-field test when compared to both the rAAV-GFP and naïve animals. In contrast to the models described earlier, these animals also showed reference learning and working memory deficits at 8 weeks after rAAV treatment when compared to the rAAV-GFP and naïve control animals (Hall et al. 2013). Taken together, the above studies show that while the use of viral vectors for the induction of aSyn overexpression in specific brain regions leads to significant cell loss in most cases, it does not always lead to the early cognitive impairments often observed in PD. In fact, it may be necessary to deliver the viral vectors to multiple brain areas implicated in PD in order to be able to observe cognitive deficits, as suggested by the most recent work from the Kirik group (Hall et al. 2013).

4 Summary and Conclusions

Mouse models of PD have traditionally focused on recapitulating the classical motor symptoms of the disease, and only a few transgenic mouse lines reliably model the early cognitive impairment in PD to serve as platforms for the testing of therapeutics targeting these deficits. Some of these models more closely resemble DLB than PD in that the observed pathology is primarily seen in the cortex and hippocampus. These mouse lines display impairments in the MWM and contextual fear memory, which are hippocampus-dependent tasks, and in cued fear memory, which is amygdala-dependent, beginning at 8–9 months of age (Freichel et al. 2007; Nuber et al. 2008; Zhou et al. 2008; Schell et al. 2009, 2012; Price et al. 2010; Lim et al. 2011). It was also observed that Aβ and aSyn could synergistically contribute to pathology (Clinton et al. 2010) and that cognitive deficits could be reversed in a

conditional model of overexpression when aSyn expression is stopped (Lim et al. 2011). Other mouse transgenic models reviewed here more closely resemble PD rather than DLB, as they display primarily brainstem and subcortical pathology, which is consistent with the pattern of pathological progression in PD (Braak et al. 2002, 2003). These mice show cognitive deficits in several different areas beginning at 4–6 months of age. Specifically, deficits were observed in the Y maze and NPR, which measure short-term spatial memory; the NOR test, which measures short-term non-spatial memory; and reversal learning, which measures cognitive flexibility (Magen et al. 2012). Although the results presented above indicate that there are differences in cognitive deficits between mice with primarily cortical pathology and those with primarily subcortical pathology, it is important to note that, in many instances, the same tasks were not examined in both types of models. As an example, many of the tests performed on the Thy1-aSyn mice were not performed on the primarily cortical models. As a result, it would be premature to confidently conclude that certain cognitive domains are more affected in one group of mice compared to the other.

There were four lines of mice that used the Thy1 promoter to overexpress wild-type, A30P, Y39C, or E57K aSyn and showed cognitive deficits, with varying ages of onset depending on the transgene that was expressed and the type of test used (Freichel et al. 2007; Zhou et al. 2008; Schell et al. 2009, 2012; Rockenstein et al. 2014). It should be noted that the Thy1-WTaSyn and Thy1-E57KaSyn models showed cognitive deficits at 4–6 and 3–4 months of age, respectively, whereas the other transgenic lines did not have significant deficits until after 12 months of age, indicating that these lines are suitable for testing drugs that improve cognition at earlier ages, which is advantageous for preclinical drug studies. All these models showed cognitive deficits without evidence of concurrent loss of nigrostriatal dopamine, suggesting that these deficits correspond to early cognitive anomalies that are detected in the premanifest phase of PD.

In addition to the traditional transgenic models of PD reviewed above, we briefly reviewed four BAC transgenic mouse lines generated in the last few years. While the Tukahashi group was able to observe decreased anxiety-like behavior and hyperlocomotion (Yamakado et al. 2012), and Li and colleagues observed motor and olfactory deficits in their experiments (Hansen et al. 2013), there have not been any reports of cognitive impairment in these novel mouse lines. These results may be due to the fact that the BAC transgenic models in most cases do not achieve sufficiently high levels of aSyn expression in the brain.

Finally, we reviewed a series of rat models using viral delivery methods to overexpress aSyn in specific brain regions. These models vary in their choice of the aSyn variant, the viral vectors used to deliver the transgene, the promoters used to drive aSyn expression, and the brain structures into which the vector is injected. While these strategies generally result in robust and localized expression of aSyn and lead to significant cell loss in the targeted areas, the only model that led to cognitive deficits utilized an rAAV vector to overexpress aSyn in 2 different brain regions (VTA and MS/vDBB) (Hall et al. 2013). This observation suggests that cell loss in any one specific brainstem area may not be sufficient to recapitulate the early

cognitive deficits observed in PD and that two or more such areas need to be targeted for this purpose.

With several notable exceptions, many mouse lines overexpressing alpha-synuclein do not replicate the nigral cell death that is observed in PD, even though more lines show decreased levels of DA in the striatum (Chesselet and Richter 2011). However, the fact that aSyn overexpression leads to early cognitive and motor deficits supports the idea that aSyn is a causative factor in PD pathogenesis. Furthermore, aSyn pathology, along with changes in the dopaminergic system and synaptic dysfunction, was observed in regions related to the impaired cognitive domains. Each of the many models discussed here exhibits a unique set of deficits that can in turn be targeted by various therapeutic strategies. For instance, in DLB-like models with primarily cortical pathology, turning off aSyn expression (Nuber et al. 2008; Lim et al. 2011) or immunization therapy (Masliah et al. 2011) has been shown to exert beneficial effects by targeting aSyn accumulation, which is hypothesized to be upstream of synaptic dysfunction resulting in cognitive deficits. Other strategies involve the targeting of specific synaptic processes. For instance, the mGluR5 antagonist MPEP was shown to have potential in treating the cognitive impairments associated with aSyn accumulation in PDGF-aSyn mice (Price et al. 2010). The early cognitive deficits in the Thy1-aSyn model, which presents with a primarily subcortical pattern of pathology, provide useful endpoint measures to test therapeutic strategies, which are currently under investigation and may lead to the development of new drugs for the treatment of the cognitive deficits observed in early Parkinson's disease.

References

Aarsland D, Kurz MW (2010) The epidemiology of dementia associated with parkinson's disease. Brain Pathol 20:633–639

Abeliovich A, Schmitz Y, Fariñas I, Choi-Lundberg D, Ho W-H, Castillo PE, Shinsky N, Verdugo JMG, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking α-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25:239–252

Amschl D, Neddens J, Havas D, Flunkert S, Rabl R, Romer H, Rockenstein E, Masliah E, Windisch M, Hutter-Paier B (2013) Time course and progression of wild type alpha-Synuclein accumulation in a transgenic mouse model. BMC Neuroscience 14:6

Beach T, Adler C, Lue L, Sue L, Bachalakuri J, Henry-Watson J, Sasse J, Boyer S, Shirohi S, Brooks R, Eschbacher J, White C III, Akiyama H, Caviness J, Shill H, Connor D, Sabbagh M, Walker D (2009) Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol 117:613–634

Botton PH, Costa MS, Ardais AP, Mioranzza S, Souza DO, da Rocha JBT, Porciúncula LO (2010) Caffeine prevents disruption of memory consolidation in the inhibitory avoidance and novel object recognition tasks by scopolamine in adult mice. Behav Brain Res 214:254–259

Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U (2002) Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 249:1–5

Braak H, Tredici KD, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211

Braak H, Rüb U, Del Tredici K (2006) Cognitive decline correlates with neuropathological stage in Parkinson's disease. J Neurol Sci 248:255–258

Brown RG, Lacomblez L, Landwehrmeyer BG, Bak T, Uttner I, Dubois B, Agid Y, Ludolph A, Bensimon G, Payan C, Leigh NP, N. S. G. (2010) Cognitive impairment in patients with multiple system atrophy and progressive supranuclear palsy. Brain 133:2382–2393

Chen PE, Specht CG, Morris RGM, Schoepfer R (2002) Spatial learning is unimpaired in mice containing a deletion of the alpha-synuclein locus. Eur J Neurosci 16:154–158

Chesselet M-F, Richter F (2011) Modelling of Parkinson's disease in mice. Lancet Neurol 10:1108–1118

Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM (2010) Synergistic Interactions between Aβ, Tau, and α-Synuclein: Acceleration of Neuropathology and Cognitive Decline. J Neurosci 30:7281–7289

Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, Hardy J, Leverenz JB, Del Tredici K, Wszolek ZK, Litvan I (2009) Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol 8:1150–1157

Farrer M, Kachergus J, Forno L, Lincoln S, Wang D-S, Hulihan M, Maraganore D, Gwinn-Hardy K, Wszolek Z, Dickson D, Langston JW (2004) Comparison of kindreds with parkinsonism and α-synuclein genomic multiplications. Ann Neurol 55:174–179

Fernagut PO, Tison F (2012) Animal models of multiple system atrophy. Neuroscience 211:77–82

Freichel C, Neumann M, Ballard T, Müller V, Woolley M, Ozmen L, Borroni E, Kretzschmar HA, Haass C, Spooren W, Kahle PJ (2007) Age-dependent cognitive decline and amygdala pathology in α-synuclein transgenic mice. Neurobiol Aging 28:1421–1435

Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T (2002) alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4:160–164

Gardai SJ, Mao W, Schüle B, Babcock M, Schoebel S, Lorenzana C, Alexander J, Kim S, Glick H, Hilton K, Fitzgerald JK, Buttini M, Chiou S-S, McConlogue L, Anderson JP, Schenk DB, Bard F, Langston JW, Yednock T, Johnston JA (2013) Elevated Alpha-Synuclein Impairs Innate Immune Cell Function and Provides a Potential Peripheral Biomarker for Parkinson's Disease. PLoS ONE 8:e71634

Geser F, Wenning GK, Poewe W, McKeith I (2005) How to diagnose dementia with Lewy bodies: State of the art. Mov Disord 20:S11–S20

Giasson BI, Duda JE, Quinn SM, Zhang B, Trojanowski JQ, Lee VMY (2002) Neuronal α-synucleinopathy with severe movement disorder in mice expressing A53T Human α-Synuclein. Neuron 34:521–533

Gurvich C, Georgiou-Karistianis N, Fitzgerald PB, Millist L, White OB (2007) Inhibitory control and spatial working memory in Parkinson's disease. Mov Disord 22:1444–1450

Hall H, Jewett M, Landeck N, Nilsson N, Schagerlöf U, Leanza G, Kirik D (2013) Characterization of cognitive deficits in rats overexpressing human alpha-synuclein in the ventral tegmental area and medial septum using recombinant adeno-associated viral vectors. PLoS ONE 8:e64844

Halliday G, Holton J, Revesz T, Dickson D (2011) Neuropathology underlying clinical variability in patients with synucleinopathies. Acta Neuropathol 122:187–204

Hansen L, Salmon D, Galasko D, Masliah E, Katzman R, DeTeresa R, Thal L, Pay MM, Hofstetter R, Klauber M, Rice V, Butters N, Alford M (1990) The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. Neurology 40:1

Hansen C, Björklund T, Petit GH, Lundblad M, Murmu RP, Brundin P, Li J-Y (2013) A novel α-synuclein-GFP mouse model displays progressive motor impairment, olfactory dysfunction and accumulation of α-synuclein-GFP. Neurobiology of Disease. 56:145–155

Hashimoto M, Rockenstein E, Masliah E (2003) Transgenic models of α-synuclein pathology. Ann NY Acad Sci 991:171–188

Ikeuchi T, Kakita A, Shiga A, Kasuga K, Kaneko H, Tan C, Idezuka J, Wakabayashi K, Onodera O, Iwatsubo T, Nishizawa M, Takahashi H, Ishikawa A (2008) Patients homozygous and heterozygous for SNCA duplication in a family with parkinsonism and dementia. Arch Neurol 65:514–519

Janezic S, Threlfell S, Dodson PD, Dowie MJ, Taylor TN, Potgieter D, Parkkinen L, Senior SL, Anwar S, Ryan B, Deltheil T, Kosillo P, Cioroch M, Wagner K, Ansorge O, Bannerman DM, Bolam JP, Magill PJ, Cragg SJ, Wade-Martins R (2013) Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. Proc Natl Acad Sci 110: E4016–E4025

Janvin CC, Larsen JP, Aarsland D, Hugdahl K (2006) Subtypes of mild cognitive impairment in parkinson's disease: Progression to dementia. Mov Disord 21:1343–1349

Kahle PJ (2008) alpha-Synucleinopathy models and human neuropathology: similarities and differences. Acta Neuropathol 115:87–95

Kahle PJ, Neumann M, Ozmen L, Müller V, Jacobsen H, Schindzielorz A, Okochi M, Leimer U, van der Putten H, Probst A, Kremmer E, Kretzschmar HA, Haass C (2000) Subcellular Localization of Wild-Type and Parkinson's Disease-Associated Mutant α-Synuclein in Human and Transgenic Mouse Brain. J Neurosci 20:6365–6373

Kao AW, Racine CA, Quitania LC, Kramer JH, Christine CW, Miller BL (2009) Cognitive and neuropsychiatric profile of the synucleinopathies: Parkinson disease, dementia with Lewy bodies, and multiple system atrophy. Alzheimer Dis Assoc Disord 23(365–370):3. doi:10.1097/WAD.1090b1013e3181b5065d

Kéri S, Nagy H, Myers CE, Benedek G, Shohamy D, Gluck MA (2008) Risk and protective haplotypes of the alpha-synuclein gene associated with Parkinson's disease differentially affect cognitive sequence learning. Genes Brain Behav 7:31–36

Kéri S, Moustafa AA, Myers CE, Benedek G, Gluck MA (2010) α-Synuclein gene duplication impairs reward learning. Proc Natl Acad Sci 107:15992–15994

Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ, Björklund A (2002) Parkinson-Like Neurodegeneration Induced by Targeted Overexpression of α-Synuclein in the Nigrostriatal System. J Neurosci 22:2780–2791

Klein RL, King MA, Hamby ME, Meyer EM (2002) Dopaminergic cell loss induced by human A30P a-synuclein gene transfer to the rat substantia nigra. Hum Gene Ther 13:605–612

Kokhan VS, Afanasyeva MA, Van'kin GI (2012) α-Synuclein knockout mice have cognitive impairments. Behav Brain Res 231:226–230

Krüger R, Kuhn W, Leenders KL, Sprengelmeyer R, Müller T, Woitalla D, Portman AT, Maguire RP, Veenma L, Schröder U, Schöls L, Epplen JT, Riess O, Przuntek H (2001) Familial parkinsonism with synuclein pathology: clinical and PET studies of A30P mutation carriers. Neurology 56:1355–1362

Kuo Y-M, Li Z, Jiao Y, Gaborit N, Pani AK, Orrison BM, Bruneau BG, Giasson BI, Smeyne RJ, Gershon MD, Nussbaum RL (2010) Extensive enteric nervous system abnormalities in mice transgenic for artificial chromosomes containing Parkinson disease-associated Î ± -synuclein gene mutations precede central nervous system changes. Hum Mol Genet 19:1633–1650

Lam HA, Wu N, Cely I, Kelly RL, Hean S, Richter F, Magen I, Cepeda C, Ackerson LC, Walwyn W, Masliah E, Chesselet M-F, Levine MS, Maidment NT (2011) Elevated tonic extracellular dopamine concentration and altered dopamine modulation of synaptic activity precede dopamine loss in the striatum of mice overexpressing human α-synuclein. J Neurosci Res 89:1091–1102

Li B, Arime Y, Hall FS, Uhl GR, Sora I (2010) Impaired spatial working memory and decreased frontal cortex BDNF protein level in dopamine transporter knockout mice. Eur J Pharmacol 628:104–107

Lim Y, Kehm VM, Lee EB, Soper JH, Li C, Trojanowski JQ, Lee VMY (2011) α-syn suppression reverses synaptic and memory defects in a mouse model of dementia with lewy bodies. J Neurosci 31:10076–10087

Lin X, Parisiadou L, Sgobio C, Liu G, Yu J, Sun L, Shim H, Gu X-L, Luo J, Long C-X, Ding J, Mateo Y, Sullivan PH, Wu L-G, Goldstein DS, Lovinger D, Cai H (2012) Conditional expression of parkinson's disease-related mutant α-synuclein in the midbrain dopaminergic

neurons causes progressive neurodegeneration and degradation of transcription factor nuclear receptor related 1. J Neurosci 32:9248–9264

Lo Bianco C, Ridet JL, Schneider BL, Déglon N, Aebischer P (2002) α-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci 99:10813–10818

Magen I, Chesselet M-F (2010) Genetic mouse models of Parkinson's disease: the state of the art. Prog Brain Res 184:53–87 (Elsevier, Chapter 4)

Magen I, Chesselet M-F (2011) Mouse models of cognitive deficits due to alpha-synuclein pathology. J Parkinson's Dis 1:217–227

Magen I, Fleming SM, Zhu C, Garcia EC, Cardiff KM, Dinh D, De La Rosa K, Sanchez M, Torres ER, Masliah E, David Jentsch J, Chesselet M-F (2012) Cognitive deficits in a mouse model of pre-manifest Parkinson's disease. Eur J Neurosci 35:870–882

Maingay M, Romero-Ramos M, Carta M, Kirik D (2006) Ventral tegmental area dopamine neurons are resistant to human mutant alpha-synuclein overexpression. Neurobiol Dis 23:522–532

Martin LJ, Semenkow S, Hanaford A, Wong M (2014) The mitochondrial permeability transition pore regulates Parkinson's disease development in mutant α-synuclein transgenic mice. Neurobiol Aging 35:1132–1152

Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders. Science 287:1265–1269

Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L (2001) β-Amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci 98:12245–12250

Masliah E, Rockenstein E, Mante M, Crews L, Spencer B, Adame A, Patrick C, Trejo M, Ubhi K, Rohn TT, Mueller-Steiner S, Seubert P, Barbour R, McConlogue L, Buttini M, Games D, Schenk D (2011) Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy Body Disease. PLoS ONE 6:e19338

McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalaria RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VM, Lees A, Litvan I, Londos E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M, DLB co. (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 65:1863–1872

Morra LF, Donovick PJ (2013) Clinical presentation and differential diagnosis of dementia with Lewy bodies: a review. Int J Geriatr Psychiatry n/a-n/a

Neumann M, Kahle PJ, Giasson BI, Ozmen L, Borroni E, Spooren W, Müller V, Odoy S, Fujiwara H, Hasegawa M, Iwatsubo T, Trojanowski JQ, Kretzschmar HA, Haass C (2002) Misfolded proteinase K-resistant hyperphosphorylated α-synuclein in aged transgenic mice with locomotor deterioration and in human α-synucleinopathies. J Clin Investig 110:1429–1439

Nuber S, Petrasch-Parwez E, Winner B, Winkler J, von Hörsten S, Schmidt T, Boy J, Kuhn M, Nguyen HP, Teismann P, Schulz JB, Neumann M, Pichler BJ, Reischl G, Holzmann C, Schmitt I, Bornemann A, Kuhn W, Zimmermann F, Servadio A, Riess O (2008) Neurodegeneration and motor dysfunction in a conditional model of parkinson's disease. J Neurosci 28:2471–2484

Oaks AW, Frankfurt M, Finkelstein DI, Sidhu A (2013) Age-dependent effects of A53T alpha-synuclein on behavior and dopaminergic function. PLoS ONE 8:e60378

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409–421

Pagonabarraga J, Kulisevsky J (2012) Cognitive impairment and dementia in Parkinson's disease. Neurobiol Dis 46:590–596

Paumier KL, Sukoff Rizzo SJ, Berger Z, Chen Y, Gonzales C, Kaftan E, Li L, Lotarski S, Monaghan M, Shen W, Stolyar P, Vasilyev D, Zaleska M, Hirst WD, Dunlop J (2013) Behavioral Characterization of A53T Mice Reveals Early and Late Stage Deficits Related to Parkinson's Disease. PLoS ONE 8:e70274

Peña-Oliver Y, Buchman VL, Dalley JW, Robbins TW, Schumann G, Ripley TL, King SL, Stephens DN (2011) Deletion of alpha-synuclein decreases impulsivity in mice. Genes Brain Behav 11:137–146

Peterson DA, Elliott C, Song DD, Makeig S, Sejnowski TJ, Poizner H (2009) Probabilistic reversal learning is impaired in Parkinson's disease. Neuroscience 163:1092–1101

Pfeiffer HCV, Løkkegaard A, Zoetmulder M, Friberg L, Werdelin L (2013) Cognitive impairment in early-stage non-demented Parkinson's disease patients. Acta Neurologica Scandinavica

Price DL, Rockenstein E, Ubhi K, Phung V, MacLean-Lewis N, Askay D, Cartier A, Spencer B, Patrick C, Desplats P, Ellisman MH, Masliah E (2010) Alterations in mGluR5 expression and signaling in Lewy Body disease and in transgenic models of alpha-synucleinopathy—implications for excitotoxicity. PLoS ONE 5:e14020

Puschmann A, Ross OA, Vilariño-Güell C, Lincoln SJ, Kachergus JM, Cobb SA, Lindquist SG, Nielsen JE, Wszolek ZK, Farrer M, Widner H, van Westen D, Hägerström D, Markopoulou K, Chase BA, Nilsson K, Reimer J, Nilsson C (2009) A Swedish family with de novo α-synuclein A53T mutation: evidence for early cortical dysfunction. Parkinsonism Relat Disord 15:627–632

Richfield EK, Thiruchelvam MJ, Cory-Slechta DA, Wuertzer C, Gainetdinov RR, Caron MG, Di Monte DA, Federoff HJ (2002) Behavioral and neurochemical effects of wild-type and mutated human α-synuclein in transgenic mice. Exp Neurol 175:35–48

Rieker C, Dev KK, Lehnhoff K, Barbieri S, Ksiazek I, Kauffmann S, Danner S, Schell H, Boden C, Ruegg MA, Kahle PJ, van der Putten H, Shimshek DR (2011) Neuropathology in mice expressing mouse alpha-synuclein. PLoS ONE 6:e24834

Robertson DC, Schmidt O, Ninkina N, Jones PA, Sharkey J, Buchman VL (2004) Developmental loss and resistance to MPTP toxicity of dopaminergic neurones in substantia nigra pars compacta of γ-synuclein, α-synuclein and double α/γ-synuclein null mutant mice. J Neurochem 89:1126–1136

Rockenstein E, Mallory M, Hashimoto M, Song D, Shults CW, Lang I, Masliah E (2002) Differential neuropathological alterations in transgenic mice expressing α-synuclein from the platelet-derived growth factor and Thy-1 promoters. J Neurosci Res 68:568–578

Rockenstein E, Nuber S, Overk CR, Ubhi K, Mante M, Patrick C, Adame A, Trejo-Morales M, Gerez J, Picotti P, Jensen PH, Campioni S, Riek R, Winkler J, Gage FH, Winner B, Masliah E (2014) Accumulation of oligomer-prone α-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain

Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, Obeso JA (2009) Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. Lancet Neurol 8:1128–1139

Rothman SM, Griffioen KJ, Vranis N, Ladenheim B, Cong W-N, Cadet J-L, Haran J, Martin B, Mattson MP (2013) Neuronal Expression of Familial Parkinson's Disease A53T α-Synuclein Causes Early Motor Impairment, Reduced Anxiety and Potential Sleep Disturbances in Mice. J Parkinson's Dis 3:215–229

Rothman SM, Griffioen KJ, Fishbein KW, Spencer RG, Makrogiannis S, Cong W-N, Martin B, Mattson MP (2014) Metabolic abnormalities and hypoleptinemia in α-synuclein A53T mutant mice. Neurobiol Aging 35:1153–1161

Schell H, Hasegawa T, Neumann M, Kahle PJ (2009) Nuclear and neuritic distribution of serine-129 phosphorylated α-synuclein in transgenic mice. Neuroscience 160:796–804

Schell H, Boden C, Chagas AM, Kahle PJ (2012) Impaired c-fos and polo-like kinase 2 induction in the limbic system of fear-conditioned α-synuclein transgenic mice. PLoS ONE 7:e50245

Senior SL, Ninkina N, Deacon R, Bannerman D, Buchman VL, Cragg SJ, Wade-Martins R (2008) Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. Eur J Neurosci 27:947–957

Siegmund A, Langnaese K, Wotjak CT (2005) Differences in extinction of conditioned fear in C57BL/6 substrains are unrelated to expression of α-synuclein. Behav Brain Res 157:291–298

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) α-synuclein locus triplication causes parkinson's disease. Science 302:841

Somme JH, Gomez-Esteban JC, Molano A, Tijero B, Lezcano E, Zarranz JJ (2011) Initial neuropsychological impairments in patients with the E46 K mutation of the α-synuclein gene (PARK 1). J Neurol Sci 310:86–89

Specht CG, Schoepfer R (2004) Deletion of multimerin-1 in α-synuclein-deficient mice. Genomics 83:1176–1178

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci 95:6469–6473

Spira PJ, Sharpe DM, Halliday G, Cavanagh J, Nicholson GA (2001) Clinical and pathological features of a Parkinsonian syndrome in a family with an Ala53Thr alpha-synuclein mutation. Ann Neurol 49:313–319

Stiedl O, Radulovic J, Lohmann R, Birkenfeld K, Palve M, Kammermeier J, Sananbenesi F, Spiess J (1999) Strain and substrain differences in context- and tone-dependent fear conditioning of inbred mice. Behav Brain Res 104:1–12

Szegő ÉM, Gerhardt E, Outeiro TF, Kermer P (2011) Dopamine-depletion and increased α-synuclein load induce degeneration of cortical cholinergic fibers in mice. J Neurol Sci 310:90–95

Taylor TN, Potgieter D, Anwar S, Senior SL, Janezic S, Threlfell S, Ryan B, Parkkinen L, Deltheil T, Cioroch M, Livieratos A, Oliver PL, Jennings KA, Davies KE, Ansorge O, Bannerman DM, Cragg SJ, Wade-Martins R (2014) Region-specific deficits in dopamine, but not norepinephrine, signaling in a novel A30P α-synuclein BAC transgenic mouse. Neurobiol Dis 62:193–207

Thiruchelvam MJ, Powers JM, Cory-Slechta DA, Richfield EK (2004) Risk factors for dopaminergic neuron loss in human α-synuclein transgenic mice. Eur J Neurosci 19:845–854

Tofaris GK, Garcia Reitböck P, Humby T, Lambourne SL, O'Connell M, Ghetti B, Gossage H, Emson PC, Wilkinson LS, Goedert M, Grazia Spillantini M (2006) Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human α-synuclein(1–120): implications for Lewy Body disorders. J Neurosci 26:3942–3950

Wakamatsu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, Kanbe D, Muramatsu S-I, Kobayashi K, Iwatsubo T, Yoshimoto M (2008a) Selective loss of nigral dopamine neurons induced by overexpression of truncated human α-synuclein in mice. Neurobiol Aging 29:574–585

Wakamatsu M, Iwata S, Funakoshi T, Yoshimoto M (2008b) Dopamine receptor agonists reverse behavioral abnormalities of α-synuclein transgenic mouse, a new model of Parkinson's disease. J Neurosci Res 86:640–646

Watson GS, Leverenz JB (2010) Profile of cognitive impairment in Parkinson's disease. Brain Pathol 20:640–645

Williams-Gray CH, Foltynie T, Brayne CEG, Robbins TW, Barker RA (2007) Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. Brain. 130:1787–1798

Winner B, Lie DC, Rockenstein E, Aigner R, Aigner L, Masliah E, Kuhn HG, Winkler J (2004) Human wild-type α-synuclein impairs neurogenesis. J Neuropathol Exp Neurol 63:1155–1166

Yamada M, Iwatsubo T, Mizuno Y, Mochizuki H (2004) Overexpression of α-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of α-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. J Neurochem 91:451–461

Yamaguchi K, Cochran E, Murrell J, Polymeropoulos M, Shannon K, Crowther RA, Goedert M, Ghetti B (2005) Abundant neuritic inclusions and microvacuolar changes in a case of diffuse Lewy body disease with the A53T mutation in the α-synuclein gene. Acta Neuropathol 110:298–305

Yamakado H, Moriwaki Y, Yamasaki N, Miyakawa T, Kurisu J, Uemura K, Inoue H, Takahashi M, Takahashi R (2012) α-Synuclein BAC transgenic mice as a model for Parkinson's disease manifested decreased anxiety-like behavior and hyperlocomotion. Neurosci Res 73:173–177

Yang XW, Gong S (2001) An overview on the generation of BAC transgenic mice for neuroscience research. Wiley, Current Protocols in Neuroscience

Yang J-H, Han S-J, Ryu JH, Jang I-S, Kim D-H (2009) Ginsenoside Rh2 Ameliorates Scopolamine-Induced Learning Deficit in Mice. Biol Pharm Bull 32:1710–1715

Yarnall AJ, Rochester L, Burn DJ (2013) Mild cognitive impairment in Parkinson's disease. Age Ageing 42:567–576

Zhou W, Milder JB, Freed CR (2008) Transgenic mice overexpressing tyrosine-to-cysteine mutant human α-synuclein: a progressive nurodegenerative model of diffuse Lewy body disease. J Biol Chem 283:9863–9870

Modeling LRRK2 Pathobiology in Parkinson's Disease: From Yeast to Rodents

Guillaume Daniel and Darren J. Moore

Abstract Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*, *PARK8*) gene represent the most common cause of familial Parkinson's disease (PD) with autosomal dominant inheritance, whereas common variation at the *LRRK2* genomic locus influences the risk of developing idiopathic PD. LRRK2 is a member of the ROCO protein family and contains multiple domains, including Ras-of-Complex (ROC) GTPase, kinase, and protein-protein interaction domains. In the last decade, the biochemical characterization of LRRK2 and the development of animal models have provided important insight into the pathobiology of LRRK2. In this review, we comprehensively describe the different models employed to understand *LRRK2*-associated PD, including yeast, invertebrates, transgenic and viral-based rodents, and patient-derived induced pluripotent stem cells. We discuss how these models have contributed to understanding LRRK2 pathobiology and the advantages and limitations of each model for exploring aspects of *LRRK2*-associated PD.

Keywords *LRRK2* · *PARK8* · Parkinson's disease · Parkinsonism · Animal model · Neurodegeneration · Dopaminergic

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G. Daniel · D. J. Moore
School of Life Sciences, Brain Mind Institute,
Ecole Polytechnique Fédérale de Lausanne (EPFL),
1015 Lausanne, Switzerland

D. J. Moore (✉)
Center for Neurodegenerative Science, Van Andel Institute, 333 Bostwick Ave NE,
Grand Rapids, MI 49503, USA
e-mail: Darren.Moore@vai.org

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1 *LRRK2* and Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative movement disorder that affects 1–2 % of individuals above 65 years of age (Lang and Lozano 1998a, b). PD is classically defined by the cardinal motor symptoms of bradykinesia, muscular rigidity, resting tremor, and postural instability, although numerous non-motor symptoms can also manifest including myriad cognitive, psychiatric, and autonomic disturbances (Jankovic 2008). Underlying the motor symptoms of PD is the relatively selective degeneration of substantia nigra pars compacta dopaminergic neurons and their projections to the caudate-putamen that results in a marked reduction of the neurotransmitter dopamine (Lang and Lozano 1998a, b). Therapies aimed at restoring dopamine (i.e., L-dopa) or dopamine-related signaling (i.e., dopamine receptor agonists) form the basis of current treatments that are initially effective at improving motor symptoms, but are palliative rather than disease—modifying. Accompanying the degeneration of dopaminergic neurons is the formation of intracytoplasmic proteinaceous inclusions in surviving brainstem neurons, termed Lewy bodies, which are enriched with fibrillar forms of the presynaptic protein α-synuclein (Spillantini et al. 1997). PD generally occurs as an idiopathic disease, although 5–10 % of cases manifest in a familial manner and to date mutations in a number of genes have been identified to unambiguously cause rare Mendelian forms of PD (Gasser 2009; Bonifati 2014). Mutations are found in the genes encoding α-synuclein (*PARK1/4*) (Polymeropoulos et al. 1997; Singleton et al. 2003), parkin (*PARK2*) (Kitada et al. 1998), DJ-1 (*PARK7*) (Bonifati et al. 2003), PTEN-induced kinase 1 (PINK1; *PARK6*) (Valente et al. 2004), leucine-rich repeat kinase 2 (LRRK2; *PARK8*) (Paisan-Ruiz et al. 2004; Zimprich et al. 2004), ATP13A2 (*PARK9*) (Ramirez et al. 2006), FBX07 (*PARK15*) (Di Fonzo et al. 2009), VPS35 (*PARK17*) (Vilarino-Guell et al. 2011; Zimprich et al. 2011), EIF4G1 (*PARK18*) (Chartier-Harlin et al. 2011), synaptojanin 1 (*SYNJ1*; *PARK20*) (Krebs et al. 2013; Quadri et al. 2013) and DNAJC6 (Edvardson et al. 2012). The identification of genetic mutations causing familial PD has provided tremendous insight into the molecular mechanisms and cellular pathways underlying neuronal degeneration.

Mutations in the *LRRK2* gene cause late-onset, autosomal dominant PD and represent the most common cause of familial PD (Biskup and West 2009).The relatively frequent G2019S mutation in *LRRK2* has also been identified in 1–2 % of idiopathic PD cases and up to 40 % of patients with familial PD depending on ethnicity (Healy et al. 2008). *LRRK2* G2019S mutation penetrance is high and

age-dependent, but incomplete, suggesting that genetic and/or environmental factors may associate with *LRRK2* to trigger dopaminergic neurodegeneration (Hulihan et al. 2008). Genome-wide association studies further indicate that common variation in the *LRRK2* gene is a risk factor for idiopathic PD (Satake et al. 2009; Simon-Sanchez et al. 2009, International Parkinson Disease Genomics et al. (2011), Lill et al. (2012). *LRRK2* mutations give rise to a late-onset form of familial PD that is clinically and neurochemically indistinguishable from idiopathic PD. Similar to idiopathic PD, brains from *LRRK2* mutant PD subjects are typically characterized by profound substantia nigra dopaminergic neurodegeneration and gliosis together with the appearance of α-synuclein-positive Lewy body pathology (Giasson et al. 2006; Ross et al. 2006). While Lewy body pathology is predominantly associated with *LRRK2* mutant PD cases, some cases reveal instead tau-positive neurofibrillary pathology, ubiquitin-positive inclusions, or even the absence of obvious pathological aggregates or inclusions (Zimprich et al. 2004; Biskup and West 2009; Crosiers et al. 2011). Therefore, *LRRK2*-associated PD shares many clinical and pathological similarities with idiopathic PD, with some minor exceptions, whereas genetically *LRRK2* variation contributes to familial and idiopathic PD.

LRRK2 is a multi-domain protein of 2,527 amino acids that belongs to the ROCO family of proteins. ROCO proteins contain a characteristic Ras-of-Complex (ROC) GTPase domain adjacent to a C-terminal-of-ROC (COR) linker region. LRRK2 also contains a serine/threonine protein kinase domain and several putative protein-protein interaction domains flanking the central ROC-COR-kinase catalytic region (Tsika and Moore 2012). LRRK2 predominantly exists as a dimeric structure and dimerization is required for its kinase activity and for its localization to cellular membranes (Greggio et al. 2008; Sen et al. 2009; Berger et al. 2010; James et al. 2012). LRRK2 is expressed at high levels in lung, kidney, and lymph nodes (Biskup et al. 2007; Westerlund et al. 2008; Hakimi et al. 2011), but also in various brain regions, including the cortex, striatum, hippocampus, cerebellum, and in the dopaminergic neurons of the SNpc, albeit at low levels (Mandemakers et al. 2012). Within the brain, LRRK2 is abundantly expressed in neurons, but can also be detected at lower levels in astrocytes and microglia where its expression can be induced by inflammatory stimuli (Moehle et al. 2012; Giesert et al. 2013). Within neurons, LRRK2 localizes to several vesicular structures and intracellular membranes (Biskup et al. 2006; Hatano et al. 2007; Alegre-Abarrategui et al. 2009) (i.e., endosomes, lysosomes, multivesicular bodies, mitochondrial outer membrane, lipid rafts, microtubule-associated transport vesicles, synaptosomes, the Golgi complex, and the endoplasmic reticulum). The structural organization and molecular function of LRRK2 are beyond the scope of this review and have been covered in detail elsewhere (Cookson 2010; Tsika and Moore 2012).

Until now, most putative substrates of LRRK2 kinase activity have been identified and validated in vitro or in invertebrate model organisms. These substrates include LRRK2 itself (Greggio et al. 2009; Webber et al. 2011; Sheng et al. 2012), MAP kinase kinase family members (Gloeckner et al. 2009; Chen et al. 2012), the ezrin/radixin/moesin (ERM) protein family (Jaleel et al. 2007; Parisiadou et al. 2009), *β*-tubulin (Gillardon 2009), Akt1 (Ohta et al. 2011), *FoxO1* (Kanao et al. 2010),

Drosophila Futsch (Lee et al. 2010b), microtubule-associated protein tau (Kawakami et al. 2012; Bailey et al. 2013), 4E-BP1 (Gehrke et al. 2010; Trancikova et al. 2012), ArfGAP1 (Stafa et al. 2012; Xiong et al. 2012), α-synuclein (Qing et al. 2009), snapin (Yun et al. 2013) and EndophilinA (Matta et al. 2012). LRRK2 autophosphorylation may serve a regulatory function and occurs at residues within or adjacent to the ROC GTPase domain (Greggio et al. 2009; Webber et al. 2011; Kamikawaji et al. 2013). The GTPase domain of LRRK2 binds guanine nucleotides and is capable of hydrolyzing GTP at a slow rate, apparently independent of its oligomerization state (Ito et al. 2007; Lewis et al. 2007; Taymans et al. 2011; Biosa et al. 2013; Liao et al. 2014). Although there is evidence for a functional interplay between the two enzymatic domains, the biochemical mechanisms governing LRRK2 enzymatic functions remain unclear. Interestingly, GTP hydrolysis and GTP binding activities of LRRK2 are both required for LRRK2 kinase activity, whereas the contribution of LRRK2 autophosphorylation within the GTPase domain to GTP binding and GTP hydrolysis activities is incompletely understood (Ito et al. 2007; West et al. 2007; Taymans et al. 2011; Biosa et al. 2013). Kinase-inactive variants of LRRK2 exhibit normal GTP binding and GTP hydrolysis activities, although mutation of individual autophosphorylation sites within the GTPase domain (i.e., T1503) can alter kinase activity (Webber et al. 2011; Biosa et al. 2013).

Although a number of familial mutations in *LRRK2* have been reported, only a few are truly pathogenic and affect highly conserved residues in various functional domains of the protein, including the ROC GTPase domain (R1441C, R1441G, R1441H), the COR linker (Y1699C), and the kinase domain (G2019S, I2020T). The G2019S mutation in the kinase domain has been shown to consistently enhance *LRRK2* kinase activity (West et al. 2005; Greggio and Cookson 2009), whereas the effect of the I2020T mutation on kinase activity is ambiguous (Gloeckner et al. 2006; Jaleel et al. 2007). Recent in vitro findings suggest that the R1441H mutation prolongs the GTP-bound state of the LRRK2 ROC domain by compromising GTPase activity and increasing GDP-GTP exchange (Liao et al. 2014). Overall, mutations in the ROC-COR domain diminish GTPase activity with little if any consistent effect on kinase activity (Lewis et al. 2007; Greggio and Cookson 2009; Xiong et al. 2010; Daniels et al. 2011; Liao et al. 2014).

In summary, pathogenic mutations of *LRRK2* are located in functional domains and alter the enzymatic activity of LRRK2, which suggests that both GTPase and kinase activity are likely to be important for LRRK2-dependent neurodegeneration in PD. Therefore, pharmacological modulation of LRRK2 enzymatic activity may comprise a promising therapeutic approach for the treatment of familial and idiopathic PD.

2 Models of *LRRK2*-Associated Parkinson's Disease

Animal models and model organisms have proven to be fundamental tools to identify and validate the molecular and cellular mechanisms underlying genetically linked disease. LRRK2 is an attractive therapeutic target for PD and insights

gained from LRRK2 animal models should help to develop and validate new therapeutic approaches for both familial and idiopathic PD. Accordingly, intense efforts have focused on the generation of LRRK2 cellular and animal models, which include simple eukaryotic organisms, invertebrates, rodents, and patient-derived neurons. These LRRK2 models and the major insights derived from them so far will be described herein.

2.1 Simple Eukaryotic LRRK2 Models: Saccharomyces cerevisiae

The baker's yeast, *Saccharomyces cerevisiae*, is commonly used in different areas of biology to unravel the molecular function(s) of proteins and the fundamental cellular processes and pathways in which they are implicated. Although lacking the physiological and genetic complexity of mammalian neurons, yeast exhibit a high degree of conservation of basic protein function and cellular pathways with mammalian cells that are implicated in neurodegenerative processes (i.e., vesicular trafficking, protein folding and aggregation, protein catabolism, mitochondrial function, etc.). In addition, the accessibility of yeast cells to genetic manipulation and genome-wide screening approaches enables the rapid identification of molecular pathways and biological processes associated with or regulated by a given protein. In the context of PD, studies in yeast have provided unique insight into the pathobiology of the α-synuclein protein and the identification of key cellular processes mediating human α-synuclein-dependent toxicity (Outeiro and Lindquist 2003; Willingham et al. 2003; Cooper et al. 2006; Gitler et al. 2008, 2009; Yeger-Lotem et al. 2009).

Yeast has been employed to investigate the mechanisms underlying the pathobiology of LRRK2, which has revealed a key role for the GTPase domain of LRRK2 in mediating cellular toxicity (Xiong et al. 2010). Overexpression of full-length human LRRK2 under the control of a galactose-inducible promoter failed to elicit cellular toxicity owing to the sequestration of LRRK2 into highly insoluble cytoplasmic inclusions (Xiong et al. 2010; Pereira et al. 2014). Xiong and coworkers focused on the effects of domain fragments of *LRRK2* on yeast viability and observed that overexpression of a large fragment containing the central ROC GTPase, COR, and kinase domains was highly cytotoxic, and that the expression of the GTPase domain alone was also sufficient to markedly reduce yeast viability. Furthermore, the introduction of GTP binding-deficient (K1347A or T1348N) variants exacerbated toxicity compared to wild-type (WT) LRRK2, whereas GTPase-hyperactive variants (R1398L or Ras-like R1398Q/T1343G) partially improved yeast viability. Importantly, however, pathogenic mutations associated with familial PD (i.e., R1441C and G2019S) do not influence the toxicity induced by human LRRK2 in yeast, which perhaps suggests that these mutations may only exert their deleterious effects in the context of full-length LRRK2 and/or in mammalian cells (Xiong et al. 2010; Pereira et al. 2014). Together, studies in yeast

support a critical role for GTPase activity in LRRK2-mediated toxicity. Defects in endocytic vesicular trafficking to the vacuole (equivalent to the mammalian lysosome) and the accumulation of autophagic vacuoles coincided with *LRRK2*-induced toxicity in yeast. Furthermore, LRRK2-induced toxicity in yeast appears to act through a mechanism distinct from toxicity induced by human α-synuclein since overexpression of the yeast proteins Ypt1 (an ortholog of mammalian Rab1) and Ykt6, which are potent suppressors of α-synuclein-induced toxicity (Cooper et al. 2006), failed to similarly alter LRRK2-mediated toxicity. A genome-wide genetic screen in yeast identified nine modifiers of LRRK2-induced toxicity, including *SLT2* and *GCS1*, which are orthologous to human MAP kinases (MAPK1, 3, 11, and 14) and ADP-ribosylation factor GTPase-activating protein 1 (ArfGAP1), respectively. ArfGAP1 is a GTPase-activating protein that plays a role in vesicular trafficking at the Golgi complex, and is critical for maintaining Golgi integrity by promoting the GTP hydrolysis of the small GTPase Arf1 (Shiba and Randazzo 2012). Subsequent studies have demonstrated a conserved interaction between LRRK2 and GCS1/ArfGAP1 in *Drosophila* (Xiong et al. 2012), as discussed later, and in mammalian cells and rodent neurons (Stafa et al. 2012). ArfGAP1 and LRRK2 proteins biochemically interact in vitro and in vivo, and ArfGAP1 serves as a GAP-like protein for LRRK2 to enhance its GTPase activity (Stafa et al. 2012; Xiong et al. 2012). Gene silencing of ArfGAP1 expression in rat primary cortical neurons rescues the impaired neurite outgrowth induced by G2019S LRRK2 (Stafa et al. 2012), similar to the suppressor effect of *GCS1* deletion in yeast (Xiong et al. 2010). LRRK2 also reciprocally phosphorylates ArfGAP1 and is required for ArfGAP1-induced neuronal toxicity (Stafa et al. 2012; Xiong et al. 2012). Therefore, the functional interaction of LRRK2 with GCS1/ArfGAP1 is conserved from yeast to mammals and may comprise an important molecular pathway for mediating LRRK2-induced neuronal toxicity.

Pereira and coworkers recently observed that low-level overexpression of full-length human WT LRRK2 in yeast confers resistance against hydrogen peroxide (H_2O_2) exposure potentially through a mechanism involving mitochondrial function and endocytosis (Pereira et al. 2014). The pathogenic G2019S and R1441C mutations, or the absence of kinase activity and the WD40 domain, abolished this resistance. Furthermore, the overexpression of WT LRRK2 modestly decreased the H_2O_2-induced production of reactive oxygen species (ROS), whereas expression of G2019S or R1441C LRRK2 oppositely stimulated ROS production, increased mitochondrial membrane potential, and induced endocytic defects in the context of H_2O_2 exposure (Pereira et al. 2014). Collectively, these observations support a role for LRRK2 in mediating protection against oxidative stress through a pathway involving mitochondrial function and endocytosis.

The baker's yeast, *Saccharomyces cerevisiae*, provides a powerful genetic and cell biological tool to dissect the fundamental biology and pathobiology of human *LRRK2*, and for the rapid identification of novel pathways that are important for *LRRK2*-mediated neuronal degeneration. Furthermore, yeast can be employed as an initial cellular model to broadly screen for chemical or genetic modifiers of LRRK2-induced toxicity prior to further validation in disease-relevant mammalian models.

2.2 *Invertebrate LRRK2 Models: Drosophila melanogaster and Caenorhabditis elegans*

2.2.1 *Drosophila melanogaster*

The fruit fly, *Drosophila melanogaster*, has proven to be a powerful model for the study of neurodegenerative diseases and in particular for investigating the function of genes associated with familial PD (i.e., α-synuclein, parkin, *PINK1*, *DJ-1*) (Chen and Feany 2005; Meulener et al. 2005; Clark et al. 2006; Park et al. 2006; Tain et al. 2009). The *LRRK2* gene is highly conserved across species. *C. elegans* and *Drosophila* each have a single paralog of human *LRRK2* and *LRRK1* (Marin 2008). Since the key residues mutated in *LRRK2*-associated familial PD are conserved between human *LRRK2* and *Drosophila LRRK* (*dLRRK*), several studies have described the generation of transgenic or mutant *Drosophila* as a model to investigate the molecular and cellular pathobiology of *LRRK2*-related PD.

Loss-of-function studies reveal that *dLRRK* is dispensable for the development and maintenance of dopaminergic neurons, but appears important for maintaining their integrity (Lee et al. 2007; Imai et al. 2008; Wang et al. 2008a). Moreover, disruption of *dLRRK* influences the sensitivity of flies to hydrogen peroxide, although it remains unclear whether dLRRK protects or sensitizes dopaminergic neurons to oxidative insult (Imai et al. 2008; Wang et al. 2008a). Homozygous *dLRRK* deletion mutants are viable into adulthood and exhibit a normal life span although while male mutants are fertile, female mutants exhibit reduced fecundity (Lee et al. 2007; Imai et al. 2008).

Although loss-of-function approaches help to clarify certain functions of *LRRK2*, they do not represent an appropriate model to investigate the pathological effects of PD-related mutations in human *LRRK2* which appear to act through a gain-of-function mechanism. Accordingly, transgenic fly models have been developed to study the impact of these familial mutations on dopaminergic neurons. Most of these studies employ the *GAL4/UAS* gene system, which relies on the transcriptional activation of an upstream-activating sequence (*UAS*) by the yeast transcriptional activator *GAL4* to express a transgene in distinct neuronal populations. Briefly, this is achieved by crossing a first transgenic responder strain where the gene of interest (dLRRK or human *LRRK2*) is inserted downstream of a genomic sequence containing multiple *GAL4* binding sites (*UAS*), with a second driver strain where *GAL4* is expressed under the control of a cell- or tissue-specific promoter (Brand and Perrimon 1993). Hence, the use of different neuronal *GAL4* drivers (i.e., *gmr*, *elav*, *TH,* or *ddc*) to drive the expression of a native or codon-optimized human *LRRK2* transgene, combined with a unique genomic integration site and variable copy number for each transgene cassette, may result in important experimental variations that can potentially explain the broad array of phenotypes observed in the different *Drosophila* LRRK2 transgenic models described to date.

Pathogenic effects of LRRK2: Transgenic flies overexpressing pathogenic forms of dLRRK or human LRRK2 recapitulate certain features of *LRRK2*-linked

PD. Collectively, the overexpression of pathogenic dLRRK, human WT LRRK2, and to a more severe extent, G2019S, Y1699C, or G2385R mutant human LRRK2, induces an adult-onset and progressive loss of dopaminergic neurons, early mortality and impaired motor function that could be attenuated by treatment with L-DOPA, and exacerbated by the mitochondrial toxin rotenone (Imai et al. 2008; Liu et al. 2008; Ng et al. 2009; Venderova et al. 2009; Liu et al. 2011; MacLeod et al. 2013). Intriguingly, a recent study demonstrated that G2019S LRRK2 expression confined to dopaminergic neurons resulted in neurodegeneration throughout the fly visual system, including within brain regions lacking obvious dopaminergic innervation (Hindle et al. 2013). G2019S LRRK2 expression in dopaminergic neurons caused a non-cell autonomous progressive loss of photoreceptor function and retinal neurodegeneration accompanied by mitochondrial dysfunction, autophagic vacuole accumulation, and apoptosis in the vicinity of photoreceptors. Expression of additional PD-associated LRRK2 mutants (I1122V, R1441C, Y1387C, Y1699C, I1915T, I2020T, and G2385R) failed to similarly impair photoreceptor function. Furthermore, the visual deficits induced by G2019S LRRK2 expression were kinase-dependent as revealed by simultaneous introduction of the kinase-inactive variant, K1906M, which disrupts the ATP-binding pocket within the kinase domain (Hindle et al. 2013). Interestingly, increasing the demands on the visual system to adapt, or increasing the activity of dopaminergic neurons, accelerates the decline in visual function induced by G2019S LRRK2 expression. These observations suggest that increased neuronal energy demand might contribute to G2019S LRRK2-mediated neurodegeneration in this fly model.

Kinase and GTPase-dependent toxic effects of LRRK2: The eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) was previously identified as a substrate of dLRRK and human *LRRK2* kinase activity that could mediate the toxic effects of LRRK2 (Imai et al. 2008). 4E-BP serves an important function for survival under starvation stress, oxidative stress, and unfolded protein stress in vivo whereby it inhibits eIF4E-mediated protein translation. Phosphorylation of 4E-BP relieves its inhibitory effect on protein translation. Familial PD mutations in dLRRK and human LRRK2 mediate the hyperphosphorylation and inactivation of 4E-BP in transgenic *Drosophila*, resulting in decreased resistance to oxidative stress, dopaminergic neuronal degeneration, and diminished climbing activity. Conversely, the overexpression of 4E-BP prevents the pathogenic effects of mutant dLRRK and attenuates neurodegeneration. This study potentially links the oxidative stress response and LRRK2 kinase activity in the context of PD. Additionally, loss of dLRRK causes the hypophosphorylation of 4E-BP and rescues dopaminergic neuronal loss in *PINK1* and *parkin* mutant fly models (Tain et al. 2009), whereas inhibitors of LRRK2 kinase activity attenuate dLRRK-mediated neurodegeneration (Liu et al. 2011). Taken together, dLRRK, through its kinase activity, is a negative regulator of dopaminergic neuronal survival.

LRRK2 has also been shown to functionally interact with the microRNA pathway to regulate protein translation. Mutant LRRK2 regulates the translation of E2F1 and DP mRNAs resulting in the overproduction of these two gene products,

which are implicated in cell cycle control and survival (Gehrke et al. 2010). LRRK2 inhibits the miRNAs let-7 and miR-184, which regulate E2F1 and DP mRNAs, respectively. Direct inhibition of the repressive activity of let-7 and miR-184 on E2F1 and DP protein synthesis could recapitulate the neurotoxic effects induced by LRRK2 in flies. Conversely, increasing the expression of let-7 or miR-184 attenuated the pathogenic effects of mutant LRRK2, suggesting that the microRNA-mediated regulation of E2F1 and DP is critical for mediating *LRRK2*-associated neurodegeneration. G2019S LRRK2 inhibits let-7 and miR-184 by interacting with and impairing the stability of *Drosophila* Argonaute-1 (dAgo1) of the RNA-induced silencing complex (RISC) in aged flies. Furthermore, G2019S LRRK2 promotes the interaction of phospho-4E-BP with dAgo1, relieving miRNA-mediated translational repression. Therefore, LRRK2-related neuronal damage could be mediated through the miRNA processing pathway, especially by regulating the translation of mRNAs.

A prior study found that dLRRK compromises dopaminergic neuronal survival in *Drosophila* through phosphorylation of *FoxO1*, a key regulator of myriad cellular processes including the cell cycle, cell death pathways, metabolism and oxidative stress, and the regulation of 4E-BP transcription. The phosphorylation of *FoxO1* by dLRRK caused an increase in expression of the pro-apoptotic proteins Bid and Him leading to activation of cell death pathways (Kanao et al. 2010). It was also shown that the overexpression of dLRRK, human WT LRRK2, and more potently, human G2019S LRRK2 induced defects in dendritic arborization (Lin et al. 2010). G2019S LRRK2 induced the redistribution and abnormal accumulation of tau in dendritic processes resulting in neurodegeneration. G2019S LRRK2 indirectly promotes the phosphorylation of tau at threonine-212 mediated by the *Drosophila* glycogen synthase kinase 3 homolog Shaggy (Sgg), which facilitates tau-dependent pathology, including microtubule fragmentation, inclusion body formation, and neuronal loss. Surprisingly, the RNAi-mediated silencing of endogenous tau expression rescued defects in dendritic arborization induced by G2019S LRRK2, whereas reduced *dLRRK* gene dosage alleviated pathogenic phenotypes induced by tau overexpression, including dendritic process degeneration, inclusion formation, and microtubule fragmentation. Collectively, these observations suggest that the functional interaction between LRRK2 and tau is rather complex and is unlikely to be mediated via a single linear pathway.

Xiong and coworkers showed that the GTPase domain of LRRK2 contributes to *LRRK2*-mediated neurodegeneration. They and others identified ArfGAP1 as a novel GAP protein that regulates the GTPase activity of *LRRK2* in vitro (Stafa et al. 2012; Xiong et al. 2012). Interestingly, although the overexpression of ArfGAP1 alone in flies induced substantial loss of dopaminergic neurons in the dorsomedial PPM1/2 cluster (equivalent to the mammalian substantia nigra), its co-expression with human WT or G2019S LRRK2 conferred protection against human *LRRK2*-induced neurotoxicity (Xiong et al. 2012). The genetic interaction between LRRK2 and ArfGAP1 appears complex, as ArfGAP1 enhances the GTP hydrolysis activity of LRRK2 in vitro and protects against LRRK2-induced dopaminergic neuronal degeneration in flies, whereas reciprocally LRRK2

phosphorylates ArfGAP1 which reduces its GAP activity in vitro and protects against ArfGAP1-mediated retinal degeneration in flies (Xiong et al. 2012). Furthermore, silencing of ArfGAP1 expression protects against LRRK2-induced neuronal toxicity in primary cultures (Stafa et al. 2012; Xiong et al. 2012), which suggests a direct role for ArfGAP1 in LRRK2-mediated neurodegeneration. However, whether ArfGAP1 is required for LRRK2-mediated neuronal toxicity in vivo in mammalian models is not yet known.

LRRK2 contributes to the homeostasis of different cellular compartments: LRRK2-induced pathology might be mediated through deregulated mitochondrial dynamics and quality control. Overexpression of G2019S LRRK2 in flight muscles and dopaminergic neurons induces marked mitochondrial pathology, and impairs locomotor activity, that can be rescued by co-expression of the PD-associated protein parkin (Ng et al. 2009). Activation of AMPK by pharmacological treatment or genetic activation could rescue dopaminergic neuronal loss and locomotor deficits, and mitigates the mitochondrial pathology induced by G2019S LRRK2 overexpression or *parkin* deletion in flies (Ng et al. 2012). Furthermore, the increased sensitivity to rotenone of LRRK2 transgenic fly models (Ng et al. 2009; Venderova et al. 2009), and the genetic interaction between *LRRK2* and other PD-associated genes, *DJ-1* and *PINK1* (Venderova et al. 2009) involved in mitochondrial homeostasis suggest that mitochondrial dysfunction could be important for LRRK2-mediated pathology.

A role for LRRK2 in dopaminergic neuronal survival could also potentially be related to its function in synaptic transmission and synaptic morphogenesis. LRRK2 controls synapse morphogenesis at the *Drosophila* neuromuscular junction through complex formation with tubulin and the microtubule (MT)-binding protein Futsch in the presynaptic compartment, and interaction with 4E-BP at the postsynaptic level (Lee et al. 2010b). Thus, *LRRK2* pathogenic mutations may impede synaptic function through deregulation of protein synthesis and MT dynamics. Additionally, EndophilinA, a presynaptic membrane-binding protein that participates in clathrin-dependent endocytosis of synaptic vesicle membranes, was recently identified as a substrate of LRRK2 kinase activity (Matta et al. 2012). G2019S LRRK2 induced the hyperphosphorylation of EndophilinA in cells and fly brain with reduced phosphorylation in *dLRRK* null flies. LRRK2-mediated phosphorylation of EndophilinA inhibits membrane tubulation and membrane association, and controls a phosphorylation cycle that regulates synaptic vesicle endocytosis (Matta et al. 2012). These observations suggest that LRRK2 regulates neurotransmission and that a tight control of LRRK2 kinase activity is required for regulating synaptic vesicle formation and endocytic function.

Finally, recent studies support a role for LRRK2/dLRRK in late endosomes, lysosomes, and the retromer complex that guide protein sorting from the endosome-lysosome pathway to the Golgi complex (Dodson et al. 2012; MacLeod et al. 2013). Dodson et al. showed that in follicle cells, dLRRK is localized to late endosomal and lysosomal membranes, where it interacts with Rab7, a key regulator of late endosomal transport. dLRRK negatively regulates the Rab7-mediated perinuclear clustering of lysosomes, whereas a mutant form of dLRRK, analogous

to the pathogenic G2019S variant, promotes lysosomal tethering and perinuclear positioning of lysosomes in a process requiring dynein and microtubules (Dodson et al. 2012). Furthermore, LRRK2 interacts with Rab7L1, a small GTPase involved in vesicular trafficking to lysosomal-related organelles and in regulating neurite process length (MacLeod et al. 2013; Beilina et al. 2014). G2019S LRRK2 overexpression altered the morphology of lysosomes and the Golgi complex that may result from defects in retromer-associated protein sorting. Co-expressing Rab7L1 or restoring retromer function by co-expressing the PD-associated protein VPS35 (Vilarino-Guell et al. 2011), a key component of the retromer complex, rescued G2019S LRRK2-mediated dopaminergic neurodegeneration in flies (MacLeod et al. 2013). Interestingly, overexpression of Rab7, the only Rab with a described role in regulation of the retromer complex, partially attenuated early lethality in G2019S LRRK2 flies relative to the more robust effects of Rab7L1 (MacLeod et al. 2013). Together, these observations suggest that impaired lysosomal activity and defective protein sorting in endosomal and lysosomal vesicular compartments may play a role in LRRK2-associated neuronal damage.

2.2.2 *Caenorhabditis elegans*

The major advantage of the nematode worm, *C. elegans*, as a model organism is the ease of conducting genetic screens and evaluating compounds or toxicants with neuroprotective or neurotoxic effects. Hence, most studies have focused on the role of LRRK2 in the response to oxidative stress, a key process implicated in PD (Wolozin et al. 2008; Saha et al. 2009; Samann et al. 2009). *LRK-1* is the *C. elegans* paralog of human *LRRK2* and *LRRK1*. LRK-1 localizes to the Golgi complex and is expressed in dopaminergic neurons of worms (Sakaguchi-Nakashima et al. 2007; Samann et al. 2009). Similar to *Drosophila* models, the effects of *LRK-1* deletion are subtle and phenotypes observed in different models are often variable.

A few studies have reported the effects of *LRK-1* deletion in worms. Sakaguchi et al. showed that deletion of *LRK-1* impaired the sorting and localization of synaptic vesicles (SV) and SV-associated proteins (Sakaguchi-Nakashima et al. 2007). LRK-1 normally excludes SV proteins from a dendritic-specific transport pathway mediated by the AP-1 clathrin adaptor, which supports a role for LRK-1 in SV-associated protein sorting to the pre-synaptic compartment of axons. Although the effects of *LRK-1* deletion on dopaminergic neuronal function or survival were not explored in this study, *LRK-1* mutants exhibit subtle defects in movement and were partially defective in chemotaxis. The loss of *LRK-1* sensitizes worms to toxicity induced by the mitochondrial Complex-I inhibitor rotenone (Wolozin et al. 2008; Saha et al. 2009) and to induction of endoplasmic reticulum stress induced by tunicamycin (Samann et al. 2009), suggesting a role for LRK-1 in cellular stress responses. The sensitivity to tunicamycin in *LRK-1* mutant worms appears to be mediated via PINK1 since its deletion suppressed the vulnerability of *LRK-1* mutants to the toxin. Oppositely, *LRK-1* deletion suppressed the enhanced sensitivity of *PINK1* mutant worms to paraquat and rescued defects in

mitochondrial cristae and impaired axonal guidance (Samann et al. 2009). LRK-1 and PINK1 act antagonistically in *C. elegans* to regulate the response to stress and neurite outgrowth. Recently, Yuan et al. showed that *LRK-1* loss-of-function in worms results in increased sensitivity of dopaminergic neurons to toxicity induced by 6-OHDA and human α-synuclein overexpression (Yuan et al. 2011) suggesting a neuroprotective effect of LRK-1. Furthermore, human LRRK2 functionally substitutes for LRK-1 to protect from human α-synuclein-induced dopaminergic neuron degeneration and LRRK2 kinase activity contributes to this neuroprotective effect. The protective effects of LRRK2 in worms require kinase activity and are mediated in part through p38 MAP kinase signaling.

Worms expressing human WT or G2019S LRRK2 exhibit reduced vulnerability to rotenone (Wolozin et al. 2008) and paraquat (Saha et al. 2009) toxicity, suggesting a protective role for LRRK2 in mitochondria and/or the oxidative stress response. In addition, overexpression of LRRK2 extended the lifespan of worms, suggesting a potentially beneficial role for LRRK2 in the aging process (Wolozin et al. 2008). Yao et al. demonstrated that overexpression of human WT, R1441C and G2019S LRRK2 in dopaminergic neurons caused age-dependent neurodegeneration, dopamine deficiency, and locomotor deficits in worms (Yao et al. 2010). In comparison to WT LRRK2, the R1441C and G2019S pathogenic variants induced more severe neurodegeneration and behavioral deficits that could be rescued by dopamine replacement. Furthermore, overexpression of K1347A LRRK2, a GTP binding-deficient mutant with impaired kinase activity, did not induce dopaminergic neurodegeneration or behavioral deficits. Yao and coworkers have also demonstrated that pharmacological inhibition of LRRK2 kinase activity rescued dopaminergic neurodegeneration and dopamine-mediated behavioral deficits in worms overexpressing R1441C or G2019S LRRK2 (Yao et al. 2013). These observations support a role for kinase activity as a critical mediator of neurotoxicity induced by R1441C and G2019S mutant LRRK2 in worm models.

2.3 Vertebrate LRRK2 Models

2.3.1 Zebrafish

Zebrafish LRRK2 (zLRRK2) protein contains all of the functional domains of human LRRK2 and displays a high degree of conservation of amino acid sequence with human LRRK2 particularly within the kinase domain (Sheng et al. 2010). zLRRK2 is strongly expressed in the brain during development and larval stages, and is expressed in muscle, ovary, gut, and most prominently in the brain of adult fish (Sheng et al. 2010). Silencing of z*LRRK2* expression using morpholinos led to severe embryonic defects and lethality. Surviving morphants displayed reduced brain size and heart edema, which could be partially rescued by human LRRK2 overexpression. Interestingly, deletion of the WD40 domain from zLRRK2 did not cause developmental defects, but instead produced PD-related phenotypes

including loss of dopaminergic neurons and locomotor defects, which could be rescued by L-DOPA treatment (Sheng et al. 2010). Furthermore, deletion of the WD40 domain led to reduced and disorganized axon tracts in the midbrain, which implicates the WD40 domain in neural development and/or neuronal maintenance. Additionally, overexpression of human LRRK2 could rescue the dopaminergic neurodegeneration in zLRRK2 ΔWD40 morphants, indicating that zLRRK2 and human LRRK2 are orthologs and that zebrafish could be considered as a relevant model to investigate the role of LRRK2 in PD and to identify therapeutic agents directed at LRRK2 (Sheng et al. 2010). Despite these promising observations, a similar study reports that deletion of the WD40 domain of zLRRK2 does not cause dopaminergic neurodegeneration (Ren et al. 2011). Furthermore, deletion of the kinase or the WD40 domain had no impact on dopaminergic neuronal survival and did not result in locomotor deficits. This latter study is consistent with prior reports of loss-of-function or knockout studies in *Drosophila* and rodents that collectively suggest a limited role for LRRK2 in dopaminergic neuron development and maintenance.

2.3.2 Rodent *LRRK2* Models

Although invertebrate models are powerful tools to screen for pharmacological or genetic modifiers of LRRK2, it is important to mention that *Drosophila* and *C. elegans* do not contain true orthologs of human *LRRK2* (Marin 2008). Comparative genomic analyses reveal that *dLRRK* and *LRK-1* are most likely paralogs of mammalian *LRRK2*. Moreover, PD is characterized by slow and progressive neurodegeneration and alteration of basal ganglia circuitry with increasing age. The absence of basal ganglia circuitry in invertebrates and their short life span make them imperfect models for studying PD. In comparison, rodent models of LRRK2 circumvent these limitations and offer a more relevant approach to understand the pathological function of LRRK2 and to validate therapeutic targets for treating idiopathic and familial PD.

LRRK2 knockout models: Andres-Mateo et al. reported that *LRRK2* knockout (KO) mice with a partial deletion of exon 39 and complete deletion of exon 40 encoding the kinase domain are viable, grossly normal, and have an intact nigrostriatal dopaminergic pathway up to 2 years of age. Furthermore, *LRRK2* KO mice do not exhibit altered sensitivity to MPTP-induced neurotoxicity (Andres-Mateos et al. 2009). *LRRK2* KO mice with a deletion of exon 2 have also been generated that similarly are viable, fertile and do not display motoric deficits (Lin et al. 2009; Tong et al. 2010, 2012). In addition, no dopaminergic neurodegeneration or α-synuclein accumulation or aggregation within the brain is detected in 20-month-old KO animals (Tong et al. 2010). Lin et al. examined the pathological interaction between LRRK2 and α-synuclein using inducible transgenic mice with expression of human A53T α-synuclein in CamKIIα-positive forebrain neurons (Lin et al. 2009). Overexpression of A53T α-synuclein led to progressive degeneration of forebrain neurons associated with motor deficits, gliosis, Golgi

fragmentation, and the somatic accumulation and aggregation of α-synuclein. The loss of *LRRK2* in these mice prevents Golgi fragmentation, α-synuclein accumulation/aggregation, microglial activation, and forebrain neuronal degeneration. The consequences of *LRRK2* deletion on motor deficits induced by A53T α-synuclein were not reported (Lin et al. 2009). In a subsequent study, Daher and colleagues employed an A53T α-synuclein transgenic mouse model under the control of the hindbrain-selective mouse prion protein (PrP) promoter to explore the pathological interaction between LRRK2 and α-synuclein (Daher et al. 2012). The deletion of *LRRK2* did not influence the lethal neurodegenerative phenotype of PrP-A53T α-synuclein transgenic mice, including premature survival, behavioral deficits, α-synuclein pathology and gliosis, suggesting that α-synuclein-mediated neurodegeneration in hindbrain neurons occurs largely independent of LRRK2 expression in mice (Daher et al. 2012). Although LRRK2 may selectively contribute to cellular aspects of α-synuclein-induced neuropathology, it is not yet clear whether inhibition of LRRK2 could be employed as a therapeutic strategy to attenuate α-synuclein-mediated neuronal damage relevant to PD.

Tong and colleagues generated two independent lines of *LRRK2* KO mice through deletion of either the promoter and exon 1 or exon 29–30 encoding the GTPase domain (Tong et al. 2010). The integrity and function of the nigrostriatal dopaminergic system was not affected in the brain of *LRRK2* null mice at 2 years of age. Neuropathological features associated with neurodegeneration, including α-synuclein or ubiquitin accumulation, gliosis or altered neuronal structure, were absent from *LRRK2* KO mice. Notably, KO mice developed age-dependent kidney abnormalities, such as reduced size due to increased apoptosis, and altered kidney morphology. KO kidneys also displayed prominent α-synuclein accumulation and aggregation, ubiquitin accumulation, and impaired activity of the autophagy-lysosomal pathway. Herzig et al. confirmed the important role of LRRK2 in the kidney and identified an additional role for LRRK2 in the lung (Herzig et al. 2011). Loss of *LRRK2* led to disrupted lysosomal homeostasis in both organs whereas no abnormalities were observed in the brain. In contrast, however, they failed to observe impaired autophagy or α-synuclein accumulation in the kidney of KO mice (Herzig et al. 2011). Recent studies suggest that the loss of *LRRK2* causes age-dependent bi-phasic alterations of autophagic activity in the kidney (Tong et al. 2012), or that *LRRK2* deletion leads to a progressive enhancement of autophagic activity in the kidney but without evidence of bi-phasic changes (Hinkle et al. 2012).

Hinkle and coworkers generated *LRRK2* KO mice by deletion of exon 41 that encodes the activation hinge of the kinase domain (Hinkle et al. 2012). At 20 months of age, *LRRK2* KO mice display no alteration of the nigrostriatal dopaminergic system and no alteration of α-synuclein or tau levels. Behavioral analysis revealed that KO mice exhibit abnormal exploratory activity in the open-field test that may indicate increased anxiety. Furthermore, although G2019S LRRK2 expression was shown to impair neurite outgrowth (MacLeod et al. 2006) and suggested to induce defects in neural stem cell proliferation and differentiation (Liu et al. 2012), the loss of *LRRK2* in mice had no effect on the spine dynamics of medium-sized spiny neurons or on neural stem cell proliferation and neuroblast cell survival in the dentate

gyrus (Hinkle et al. 2012). Paus et al. have examined adult neurogenesis and the dendritic morphology of adult newborn neurons in the dentate gyrus of *LRRK2* KO mice (Paus et al. 2013). The proliferation and survival of neural precursors is not altered in KO mice. However, the loss of *LRRK2* increases the number of double-cortin-positive migrating neuroblasts and immature neurons, although the total number of mature neurons remains unaltered. Furthermore, immature neuroblasts in KO mice displayed enhanced dendritic branching and complexity while the density of mossy fibers projecting from the dentate gyrus to the hippocampal CA3 region was increased in KO mice (Paus et al. 2013). Collectively, these studies suggest a regulatory role for LRRK2 in adult neurogenesis by modulating neural stem cell fate and by shaping dendritic branching and the axonal output of adult newborn neurons in the hippocampus.

Recently, two *LRRK2* KO rat models have been developed (Baptista et al. 2013; Ness et al. 2013). *LRRK2* KO rats displayed significant weight gain compared to wild-type rats together with morphological and histopathological alterations in kidney, liver and lung, changes in the cellular composition of the spleen, modification of serum chemistry, and subtle differences in the response to immunologic challenge. However, the consequences of *LRRK2* deficiency in the brain were not reported in these studies (Baptista et al. 2013; Ness et al. 2013). In summary, mice and rats with disruption of *LRRK2* display similar alterations in kidney homeostasis suggesting that the function of LRRK2 in the kidney may be conserved across species.

Classic LRRK2 transgenic models: Inducible transgenic mice overexpressing human LRRK2 were first described by Lin and colleagues (Wang et al. 2008b; Lin et al. 2009; Parisiadou et al. 2009). Transgenic mice expressing HA-tagged human LRRK2 under the transcriptional control of a tetracycline operator (TetO)-regulated promoter were crossed with transgenic mice expressing a Tet transactivator (tTA) from the CamKIIα promoter. In vitro studies demonstrated that primary neurons derived from G2019S LRRK2 mice display reduced axonal outgrowth and identified a role for LRRK2 in neuronal morphogenesis through F-actin remodeling (Wang et al. 2008b; Parisiadou et al. 2009). WT, G2019S and kinase domain-deficient (KD) LRRK2 transgenic mice are viable and develop normally, whereas G2019S LRRK2 lines display increased ambulatory activities starting at 12 months of age (Lin et al. 2009). No signs of neurodegeneration or neuropathology were detected in WT and G2019S LRRK2 transgenic lines at 12 and 20 months of age. Expression of WT and G2019S LRRK2 altered microtubule organization and induced Golgi fragmentation apparently through a kinase-independent mechanism (Lin et al. 2009). Despite the limited pathology in these LRRK2 transgenic mice, the overexpression of WT or G2019S LRRK2 accelerated the progression of A53T α-synuclein-mediated neuropathology and neurodegeneration in the forebrain of conditional transgenic mice (Lin et al. 2009). LRRK2 overexpression also exacerbated the toxic cellular effects of A53T α-synuclein in forebrain neurons, including Golgi fragmentation, impairment of the ubiquitin-proteasome system, and mitochondrial abnormalities (Lin et al. 2009). Collectively, this study provides support for the pathological interaction of LRRK2 and α-synuclein in PD.

The contribution of LRRK2 to regulating α-synuclein-related neuropathology could be specific to certain neuronal populations or brain regions. We and others do not observe a pathological interaction between human LRRK2 and α-synuclein when employing alternative A53T α-synuclein transgenic mice driven by the hindbrain-selective PrP or broadly expressing Thy1 promoters (Daher et al. 2012; Herzig et al. 2012). Both studies demonstrate that high levels of G2019S LRRK2 expression within neurons of the cortex, striatum, brainstem, and spinal cord of mice do not exacerbate α-synuclein-mediated neuropathology. Herzig et al. established transgenic mice expressing human WT LRRK2 or G2019S LRRK2 under the control of a Thy1 promoter, which directs widespread expression in neurons of the cortex, brainstem, and spinal cord (Herzig et al. 2012). LRRK2 transgenic expression did not induce motoric abnormalities nor altered levels of endogenous tau and α-synuclein in the brain at 15 months of age (Herzig et al. 2012). Daher and colleagues showed that expression of G2019S LRRK2 has a limited impact on the lethal neurodegenerative phenotype that develops in A53T α-synuclein transgenic mice, including premature lethality, behavioral deficits, and human α-synuclein or glial neuropathology (Daher et al. 2012). Furthermore, the co-expression of A53T α-synuclein and G2019S LRRK2 did not combine to induce nigral dopaminergic neuronal loss in this model (Daher et al. 2012). At present, it is not clear whether LRRK2 consistently enhances α-synuclein-related neuropathology in mice, or whether the pathological interaction of these two proteins is restricted to specific neuronal populations.

Ramonet and coworkers developed LRRK2 transgenic mice bearing the PD-associated R1441C or G2019S mutations (Ramonet et al. 2011). In this model, human LRRK2 transgenes are expressed under the transcriptional control of a hybrid CMV-enhanced human platelet derived growth factor β-chain (CMVe-PDGFβ) promoter. G2019S LRRK2 was highly expressed in many brain areas, including the olfactory bulb, cerebral cortex, striatum, hippocampus, and cerebellum and at lower levels in substantia nigra dopaminergic neurons, whereas surprisingly R1441C LRRK2 expression was restricted to the cerebral cortex and cerebellum (Ramonet et al. 2011). Overexpression of G2019S LRRK2 leads to the progressive and selective degeneration of nigrostriatal dopaminergic neurons ($\sim$20 %) up to 2 years of age. At 14–15 months of age, no alteration in striatal dopamine levels or locomotor activity could be detected in G2019S LRRK2 mice. Unexpectedly, R1441C LRRK2 transgenic mice exhibit impaired locomotor activity accompanied by reduced catecholamine levels in the cerebral cortex consistent with the restricted pattern of transgene expression (Ramonet et al. 2011). In vitro studies revealed that cultured primary midbrain dopaminergic neurons derived from G2019S LRRK2 mice exhibit reduced neurite complexity. G2019S LRRK2 mice failed to develop α-synuclein, ubiquitin or tau neuropathology or gliosis. Notably, G2019S or R1441C LRRK2 expression resulted in the accumulation of autophagic vacuoles and damaged mitochondria in the brains of aged mice (Ramonet et al. 2011). Collectively, this model demonstrates that the common G2019S mutation exerts deleterious effects on the nigrostriatal dopaminergic pathway in mice possibly involving abnormal autophagy.

Chen et al. generated similar transgenic mice expressing HA-tagged human WT or G2019S LRRK2 from the same CMV-enhanced PDGFβ promoter. The G2019S LRRK2 mice also display a progressive, late-onset degeneration of substantia nigra dopaminergic neurons (Chen et al. 2012). In addition, G2019S LRRK2 expression increased tau phosphorylation, whereas the levels of ubiquitin or α-synuclein were unaltered. G2019S LRRK2 mice exhibit decreased striatal dopaminergic fiber density accompanied by reduced dopamine reuptake in the striatum. Furthermore, G2019S LRRK2 mice displayed L-DOPA-responsive progressive motor deficits. Interestingly, a comparative phosphoproteomic analysis between WT and G2019S LRRK2 brains revealed that MAP kinase kinase 4 (MKK4) could be a potential substrate of LRRK2 kinase activity in the substantia nigra (Chen et al. 2012). G2019S LRRK2 mice displayed increased MKK4 phosphorylation at 12 months of age, which correlated with abnormal activation of the MKK4-JNK-c-Jun-mediated cell death pathway. Collectively, this transgenic model could recapitulate some of the key features of PD subjects harboring the G2019S mutation and suggests a potential role for MKK4 phosphorylation in G2019S LRRK2-mediated dopaminergic neurodegeneration (Chen et al. 2012).

Maekawa and coworkers generated transgenic mice constitutively expressing V5-tagged human I2020T LRRK2 from a CMV promoter (Maekawa et al. 2012). I2020T LRRK2 was expressed at $\sim$1.5 fold the level of endogenous LRRK2 in all brain areas examined, including the striatum and substantia nigra. I2020T LRRK2 mice are viable and exhibit normal weight and fertility. Furthermore, expression of I2020T LRRK2 had no influence on nigral dopaminergic neuronal number or striatal dopaminergic fiber density. I2020T LRRK2 mice display a transient impairment of locomotor activity, reduced striatal dopamine content, fragmented Golgi apparatus, and an elevated degree of tubulin polymerization, which was not mediated through increased tau phosphorylation. Primary dopaminergic neurons derived from the ventral midbrain of I2020T LRRK2 mice display increased vulnerability in vitro and reduced neurite length.

Zhou et al. reported the first rat transgenic model expressing G2019S LRRK2 (Zhou et al. 2011). They created constitutive and inducible lines to temporally control G2019S LRRK2 expression. Constitutive expression of LRRK2 in rats failed to induce any behavioral phenotype, whereas the conditional adult-onset expression of LRRK2 caused abnormal locomotor activity in aged animals possibly through altered striatal dopamine reuptake. Despite this behavioral alteration, LRRK2 expression had no effect on the number of dopaminergic and noradrenergic neurons or on striatal dopamine content. No inclusions positive for α-synuclein, ubiquitin, or phosphorylated tau were detected in the brains of G2019S LRRK2 rats. Hence, inducible LRRK2 transgenic rats manifest early dopaminergic neuronal dysfunction potentially akin to asymptomatic subjects carrying the G2019S mutation (Zhou et al. 2011).

Bacterial artificial chromosome (BAC) LRRK2 transgenic models: One of the criticisms of classical transgenic models that employ mini-gene cassettes relates to the non-physiological levels of transgene expression in cell populations that do not closely reflect the normal endogenous pattern of gene expression. Furthermore,

chromosome-position effects resulting from the random integration of small transgenes within the host genome can often result in transgenic founder animals with distinct transgene expression levels and patterns that may account for the diverse phenotypes observed among different mouse models. Models that employ bacterial artificial chromosome (BACs) constructs circumvent many of these issues as they utilize the endogenous promoter and regulatory elements to recapitulate the normal expression pattern of a (trans)gene of interest, and are less susceptible to chromosome-position effects at the genomic integration site owing to their large size (~150–200 Kb) and nature. Furthermore, BAC transgenic constructs typically integrate within the genome at lower copy number (~1–10 copies) than mini-gene cassettes, thereby more closely recapitulating expression levels of the endogenous gene. This, however, can also be a disadvantage as in many cases high non-physiological levels of transgene expression are required to precipitate robust neurological phenotypes within the life span of mice.

Li and colleagues described the first BAC transgenic mice expressing human WT or R1441G LRRK2 using a BAC clone containing the human *LRRK2* genomic sequence (Li et al. 2009). R1441G LRRK2 was expressed in the cortex, cerebellum, striatum, and ventral midbrain at 5–10 fold the level of endogenous LRRK2. WT and R1441G LRRK2 mice develop normally without alteration in body or brain weight. R1441G LRRK2 mice displayed age-dependent and progressive L-DOPA-responsive motor deficits typified by impaired vertical rearing activity and akinesia at 10–12 months of age, accompanied by a modest reduction of striatal dopamine release. R1441G LRRK2 expression did not affect the number of nigrostriatal dopaminergic neurons or their striatal nerve terminals in mice at 9–10 months of age, although a modest reduction in cell body size and the density of TH-positive dendrites of nigral dopaminergic neurons was observed. Furthermore, some evidence of dopaminergic axonal damage, including axonal varicosities, spheroids, and dystrophic neurites, could be detected in the striatum and piriform cortex that are enriched with dopaminergic projections. R1441G LRRK2 BAC mice did not display alterations in α-synuclein and ubiquitin, whereas neuronal processes positive for hyperphosphorylated tau were detected in the striatum and cortex. It is not clear how the subtle dopaminergic neuropathology of R1441G LRRK2 mice precipitates the profound motor deficits in this BAC model, but these observations suggest either dysfunction of nigrostriatal dopaminergic neurotransmission or an extra-nigral origin of the dopamine-dependent motor deficits such as from the prefrontal cortex. Further dissection and validation of this motor phenotype is required especially since two recent studies could not replicate the original motor deficits in this BAC model that were initially evident at 10 months of age. Dranka et al. reported that the same R1441G LRRK2 BAC mice alternatively display deficits in motor coordination in the Rotarod and pole tests at 16 months of age (Dranka et al. 2013), whereas Bichler et al. reported that R1441G LRRK2 mice developed mild hypokinesia in the open-field arena at 16 months with gastrointestinal dysfunctions beginning at 6 months (Bichler et al. 2013). R1441G LRRK2 mice do not additionally display non-motor phenotypes,

including depression and anxiety-like behaviors, altered pain sensitivity and olfaction, or impaired learning and memory (Bichler et al. 2013).

Additional LRRK2 BAC transgenic mouse models have also been developed (Li et al. 2007, 2010). Li and coworkers generated transgenic mice using a BAC clone encompassing the entire mouse *LRRK2* (WT and G2019S) genomic sequence. FLAG-tagged WT and G2019S LRRK2 transgenes were expressed at similar levels in the cerebral cortex, ventral tegmental area, amygdala, and hippocampus, and could be detected in substantia nigra dopaminergic neurons. WT or G2019S LRRK2 expression did not affect nigrostriatal dopaminergic neuron survival or nerve terminal morphology in mice at 20 months of age. WT LRRK2 BAC mice displayed a reduced number of phospho-tau-positive cells in the striatum compared to control or G2019S LRRK2 mice, suggesting that LRRK2 might prevent the accumulation of phosphorylated tau in the brain (Li et al. 2010). However, the significance of this tau phenotype is unclear since wild-type mice do not normally contain detectable hyperphosphorylated tau in the brain. WT and G2019S LRRK2 transgenic expression altered striatal dopamine transmission in an opposite manner. WT LRRK2 mice had enhanced striatal dopamine release with unaltered dopamine uptake or tissue content, and accordingly WT LRRK2 mice were hyperactive and showed enhanced motor performance. Conversely, G2019S LRRK2 mice showed normal motor function, but displayed an age-dependent decrease in striatal dopamine content and decreased striatal dopamine release (Li et al. 2010). Collectively, these BAC mice reveal that LRRK2 regulates dopaminergic neurotransmission in the striatum and contributes to the control of motor activity.

Melrose et al. also developed BAC mice expressing human WT and G2019S LRRK2 (Melrose et al. 2010). LRRK2 transgene and endogenous LRRK2 expression were similar throughout the brain, except in the hippocampus, where transgene expression was higher. Expression of LRRK2 had no influence on nigral dopaminergic neuron number but correlated with reduced extracellular dopamine levels in the striatum. In contrast to R1441C knockin mice (see below; Tong et al. 2009), decreased dopamine levels in these BAC mice were not caused by alteration of D2 autoreceptor-mediated inhibition of dopamine synthesis and release (Melrose et al. 2010). G2019S LRRK2 mice display normal sensorimotor function but exhibit reduced exploratory behaviors, which may reflect increased anxiety. Pathologically, G2019S LRRK2 mice display an age-dependent increase in tau levels and phosphorylation suggesting altered tau metabolism (Melrose et al. 2010). G2019S LRRK2 BAC mice also exhibit reduced adult neurogenesis, which could be partially rescued by physical exercise (Winner et al. 2011). G2019S LRRK2 expression altered the proliferation and migration of neuroblasts in neurogenic niches of the adult brain, and exerted a negative impact on neurite outgrowth and spine density of hippocampal newborn neurons.

In summary, BAC transgenic models expressing LRRK2 mutations exhibit a consistent yet mild neuropathological phenotype characterized by dysfunction of nigrostriatal dopaminergic neurotransmission, altered locomotor activity, and increased tau expression and/or phosphorylation. These phenotypes could potentially represent the earliest derangements of the nigrostriatal dopaminergic

pathway in PD. BAC LRRK2 models fail, however, to recapitulate other cardinal features of PD, including dopaminergic neuronal degeneration (or indeed any evidence of neuronal loss) and α-synuclein accumulation and aggregation. BAC LRRK2 mice could therefore be considered an early pre-symptomatic model of PD prior to manifestation of neuronal degeneration and associated motor deficits. These mild phenotypes may result from insufficient levels of LRRK2 transgene overexpression in nigral dopaminergic neurons compared to some classical transgenic models that exhibit neurodegeneration (Ramonet et al. 2011; Chen et al. 2012). Collectively, studies in BAC mice support a role for LRRK2 in the regulation of striatal dopaminergic neurotransmission and motor control.

LRRK2 knockin mice: Tong and colleagues generated LRRK2 R1441C knockin (KI) mice by introducing the R1441C mutation into exon 31, thereby allowing its expression under the control of endogenous regulatory elements (Tong et al. 2009). R1441C LRRK2 KI mice are viable, fertile, and appear grossly normal. The R1441C mutation had no impact on dopaminergic neuron number or morphology in the substantia nigra, or upon noradrenergic neurons in the locus coeruleus. Striatal dopamine levels and dopamine turnover are normal in R1441C KI mice. Gliosis and the accumulation or abnormal phosphorylation of α-synuclein, ubiquitin, and tau are not altered in 22-month-old KI mice. R1441C KI mice displayed normal spontaneous locomotor activity, but exhibited impaired amphetamine-stimulated locomotor activity, altered dopamine D2 receptor-mediated function in the striatum and reduced catecholamine release in cultured chromaffin cells (Tong et al. 2009). Together, the R1441C mutation in mice impairs stimulated nigrostriatal dopaminergic transmission and D2 receptor function.

Liu and colleagues developed R1441G LRRK2 KI mice and investigated the effects of the R1441G mutation on the nigrostriatal dopaminergic pathway (Liu et al. 2014). R1441G KI mice are viable, fertile, and have normal body weight, brain size, and locomotor activity. R1441G KI mice do not display any alteration in the number and morphology of substantia nigra dopaminergic neurons or the density of striatal dopaminergic nerve terminals. Alterations in autophagy or abnormal α-synuclein, tau, or ubiquitin aggregation or accumulation could not be detected in the brains of R1441G KI mice. R1441G KI mice do exhibit an increased vulnerability to, and slower recovery from, reserpine-induced synaptic dopamine depletion and locomotor impairment (Liu et al. 2014). Collectively, observations in R1441C and R1441G LRRK2 KI mice indicate that pathogenic mutations in the ROC GTPase domain cause striatal dopaminergic synaptic vulnerability and perturbed nigrostriatal dopaminergic neurotransmission.

Herzig and coworkers generated G2019S LRRK2 KI mice (Herzig et al. 2011). In contrast to *LRRK2* KO mice, G2019S KI mice do not display any morphological alterations in kidney and lung tissues, suggesting that the G2019S mutation does not manifest a loss-of-function, but KI mice exhibit increased diastolic pressure (Herzig et al. 2011). Analysis of G2019S KI mouse brain revealed that the G2019S mutation does not cause any remarkable neuropathology and had no influence on the nigrostriatal dopaminergic pathway. Furthermore, G2019S KI mice display

normal drug-induced locomotor activity (Herzig et al. 2011). Collectively, the G2019S mutation in mice has minimal impact on the nigrostriatal dopaminergic system, suggesting that LRRK2 KI mice do not represent robust models of PD.

Viral-mediated gene transfer of LRRK2 in rodents: Viral-mediated gene delivery direct to the rodent brain offers several advantages over the conventional transgenic rodents outlined above, including their simple and rapid generation compared to transgenic animals, the possibility to deliver the transgene during adulthood to avoid potential developmental compensatory effects, and the capacity to directly compare multiple variants of the same transgene with equivalent expression levels and patterns (Low and Aebischer 2012). Gene delivery of viral vectors also permits direct targeting of specific neuronal populations that may not be readily accessible using transgene cassettes with defined promoter elements in mice. In addition, whereas transgenic models are typically limited to mice and rats, viral models can be developed in rodents and non-human primates, which serve to hasten translation to human diseases.

In the context of PD, viral models offer additional advantages. First, investigating the specific effects of transgene expression in substantia nigra dopaminergic neurons can be achieved through direct stereotactic injection. Despite a limited diffusion of the virus to adjacent regions, the transgene expression remains localized to the targeted area, and the viral serotype or promoter element can be altered to improve and optimize neuronal-specific transgene expression. Conversely, a strict control of transgene expression is difficult to achieve in transgenic rodents whereby widespread transgene expression can often lead to confounding extra-nigral phenotypes, whereas alternatively it has also proved difficult to achieve sufficient levels of transgene expression in nigral dopaminergic neurons using available promoter elements. Furthermore, the unilateral injection of viral vectors into one hemisphere of the brain allows one to determine the impact of transgene expression on dopaminergic neuron survival and physiology by direct comparison to the non-injected, unaltered hemisphere of the same animal. High-level transgene expression can also be achieved, which will help to accelerate the onset and progression of dopaminergic neurodegeneration. This aspect may be critical since neurodegeneration can typically be observed a few weeks after viral delivery to the brain, whereas transgenic models require substantial time before neuronal loss (if any) becomes apparent. Additionally, the integration and copy number of transgene per cell can be modulated by adjusting the viral titer injected, which allows one to experimentally correlate phenotype severity with transgene dosage. Finally, viral-mediated gene transfer models can be easily applied to existing environmental and/or genetic animal models of PD to study genetic or pathological interactions, or to validate therapeutic targets.

Adeno-associated viral (AAV)-based models of α-synuclein-induced toxicity in nigral dopaminergic neurons have been successfully established as important models of PD (Kirik et al. 2002; Klein et al. 2002; Lo Bianco et al. 2002; Low and Aebischer 2012). Due to the limited packaging capacity of AAV vectors, similar approaches could not be used to deliver the human *LRRK2* gene into midbrain dopaminergic neurons. Two rodent models of viral-mediated LRRK2 expression have so far been reported. Lee et al. developed a herpes simplex virus (HSV)

amplicon-based mouse model of G2019S LRRK2-induced dopaminergic neurotoxicity, whereas Dusonchet et al. generated a rat model of progressive dopaminergic neurodegeneration using a second-generation human adenovirus serotype 5 expressing human G2019S LRRK2 (Lee et al. 2010a; Dusonchet et al. 2011).

The model described by Lee and coworkers is based upon a single unilateral striatal injection of HSV expressing a CMV-driven GFP reporter and untagged human LRRK2 from the immediate-early 4/5 gene promoter (Lee et al. 2010a). Injection of HSV was performed in the striatum to avoid non-specific inflammatory damage to the substantia nigra and resulted in the transduction of ~75 % of the dopaminergic neurons in the ipsilateral nigra. The nigrostriatal expression of WT LRRK2 induced modest nigral dopaminergic neurodegeneration (~10–20%), whereas expression of the kinase-hyperactive G2019S LRRK2 resulted in ~50% neuronal loss in the ipsilateral nigra associated with reduced striatal dopaminergic fiber density at 3 weeks post-injection. Furthermore, expression of a kinase-inactive variant, G2019S-D1994A, which abolished the elevated kinase activity of the familial G2019S mutation, did not induce dopaminergic neuronal loss (Lee et al. 2010a). Collectively, this study confirms prior findings in G2019S LRRK2 transgenic mice (Ramonet et al. 2011; Chen et al. 2012) and strongly supports a critical role for elevated kinase activity in mediating the G2019S LRRK2-dependent degeneration of dopaminergic neurons in mice (Lee et al. 2010a). To further validate the kinase activity of LRRK2 as a potential therapeutic target for LRRK2-related PD, the protective effects of pharmacological inhibition of LRRK2 kinase activity were evaluated in this HSV model. Using a library of kinase inhibitors, two potent inhibitors of LRRK2 kinase activity (GW5074 and indirubin-3′-monooxime) were identified in vitro (Lee et al. 2010a). The twice daily intraperitoneal injection of either inhibitor in mice injected with HSV-LRRK2 G2019S attenuated dopaminergic neuronal degeneration. Although these findings are promising, it is not clear from this study whether these kinase inhibitors act directly on LRRK2 since both compounds are selective for and more potently inhibit a number of additional kinases. It will be important to further evaluate this HSV LRRK2 rodent model using highly selective, potent, and brain-penetrant LRRK2 kinase inhibitors that have recently been developed (Choi et al. 2012; Reith et al. 2012; Sheng et al. 2012). Altogether, this study suggests that pharmacological inhibition of LRRK2 kinase activity is a promising therapeutic approach for the treatment of neurodegeneration in *LRRK2*-associated PD. Despite the promising neurodegenerative phenotype, the authors do not describe the behavioral or cytopathological consequences of G2019S LRRK2 expression in the nigrostriatal pathway (Lee et al. 2010a).

In a second study, Dusonchet and colleagues generated a LRRK2 model based on the unilateral injection of recombinant, second-generation human serotype 5 adenoviral (rAd) vectors expressing FLAG-tagged human WT or G2019S LRRK2 driven by a neuronal-specific human synapsin-1 promoter (Dusonchet et al. 2011). Injections of the rAd vectors were performed at 6 distinct sites in the striatum of adult rats as adenoviral particles undergo efficient retrograde axonal transport to dopaminergic neurons of the substantia nigra, whereas direct injections into the

nigra result in poor transduction efficiency. At 10 days post-injection, ~30 % of nigral dopaminergic neurons exhibited strong transgene expression that persisted up to 42 days albeit with a progressive reduction in expression over time. Despite the moderate proportion of dopaminergic neurons transduced, the expression of human LRRK2 was 2-fold greater than endogenous LRRK2 in the substantia nigra suggesting that infected cells express high levels of the transgene. WT LRRK2 or GFP did not cause dopaminergic neurodegeneration, whereas G2019S LRRK2 expression induced the progressive loss of ~20% of dopaminergic neurons in the ipsilateral substantia nigra over 42 days, but without a corresponding reduction of striatal dopaminergic fiber density (Dusonchet et al. 2011). The expression of WT and G2019S LRRK2 was transiently associated with the abnormal hyperphosphorylation of tau in dystrophic dopaminergic neuritic processes, thereby uncoupling tau pathology from neurodegeneration. However, the accumulation or aggregation of ubiquitin or α-synuclein could not be detected.

Collectively, this study demonstrates the feasibility of developing viral models of LRRK2-mediated neurodegeneration in rodents and provides strong in vivo support for the contribution of elevated kinase activity to LRRK2-dependent neurotoxicity. Interestingly, the correlation between LRRK2 expression levels and tau pathology recapitulates observations in some G2019S *LRRK2* PD subjects and suggests that this viral model may be useful for exploring the interaction between LRRK2 and tau in PD. However, the limited retrograde transport of adenovirus to the substantia nigra together with a progressive reduction of transgene expression over time makes this model unsuitable for long-term assessments of the pathological effects of G2019S LRRK2 expression in vivo.

Recently, Beilina and coworkers demonstrated that LRRK2 forms a protein complex with Rab7L1, Cyclin-G-associated kinase (GAK), and Bcl2-associated athanogene 5 (BAG5) to promote the clearance of *trans*-Golgi network (TGN)-derived vesicles (Beilina et al. 2014). Pathogenic mutations in LRRK2 that increase kinase activity or disrupt GTPase activity showed an enhanced clearance of the Golgi in cultured cells. To corroborate these findings in vivo, the authors injected lentiviral vectors expressing GFP-tagged G2019S LRRK2 in the striatum of adult mice. At 2 weeks post-injection, the viral-mediated expression of G2019S LRRK2 caused a marked reduction in GLG1 immunoreactivity, a TGN marker, suggesting a potential role for LRRK2 in TGN turnover (Beilina et al. 2014). Prior studies have also described perturbations to Golgi morphology induced by G2019S LRRK2 expression (Lin et al. 2009; Stafa et al. 2012). It is not yet clear whether increased TGN turnover is specific for G2019S LRRK2 compared to WT or other variants of LRRK2, involves kinase activity, selectively occurs in particular neuronal populations, or is required for LRRK2-dependent neuronal degeneration. Nevertheless, the impact of mutant LRRK2 on Golgi-mediated vesicular trafficking could provide a promising avenue for future investigations.

2.3.3 *LRRK2*-Induced Pluripotent Stem Cells (iPSc)

In 2007, the first human-induced pluripotent stem cells (iPSc) were described providing a new approach to studying human disease (Takahashi et al. 2007; Yu et al. 2007). Prior translational research efforts were based on expression of human genes in immortalized cell lines, primary cultures, or animal models. Now, it is possible to investigate the consequences of genetic mutations in several patient-derived cell subtypes whereby the genome and its transcriptional control are mostly intact. Compared to animal models, iPSc models allow one to study the consequences of genetic mutations directly on human cellular physiology and therefore provide an important yet complementary model in which to understand disease mechanisms. However, like any cultured cell, iPS cells provide limited information and cannot recapitulate the complexity of brain circuits and the physiological diversity of neuronal populations of the intact mammalian brain. Nevertheless, iPSc provide useful disease-relevant cellular models that incorporate human genetic diversity. In PD, iPSc can provide highly relevant models because of the clear involvement of nigrostriatal dopaminergic neurons in disease and the well-developed capacity to generate iPSc-derived dopaminergic neurons.

The first models of PD developed from iPSc were derived from idiopathic PD subjects due to limited accessibility to fibroblasts from PD subjects with familial mutations (Park et al. 2008; Soldner et al. 2009). Nguyen et al. described the first monogenic PD model using iPSc derived from a skin biopsy of a 60-year-old female patient with early-onset and typical L-DOPA-responsive PD harboring a homozygous G2019S mutation in *LRRK2* (Nguyen et al. 2011). iPS cells were expanded for 8 months and a small proportion of cells could be differentiated into TH-positive dopaminergic neurons ($\sim$3.6–5%). G2019S *LRRK2* iPSc-derived TH-positive neurons selectively exhibited accumulation of α-synuclein, up-regulation of key oxidative stress response genes and increased vulnerability to neurotoxins, including hydrogen peroxide, MG132, and 6-OHDA. Sánchez-Danés and coworkers generated dopaminergic neurons from four G2019S *LRRK2* PD subjects (Sanchez-Danes et al. 2012). Following long-term culture, G2019S *LRRK2* iPSc-derived dopaminergic neurons displayed α-synuclein accumulation, altered morphology with fewer and shorter neurites, and compromised autophagosome maturation suggesting deficits in the autophagy pathway. Therefore, G2019S *LRRK2* iPS cells could recapitulate some pathological features of LRRK2 transgenic animal models and idiopathic and *LRRK2*-associated PD, thereby validating them as potential models of PD.

Cooper et al. generated iPSc-derived neural cells from subjects carrying homozygous G2019S mutations or heterozygous R1441C substitutions in *LRRK2* (Cooper et al. 2012). This study observed that iPSc-derived *LRRK2* neural cells display alterations in cellular basal oxygen consumption, mitochondrial dynamics and morphology, and increased vulnerability to a number of cellular stressors known to induce mitochondrial dysfunction. Furthermore, co-treatment with rapamycin or LRRK2 kinase inhibitors increased the resistance of *LRRK2* neural cells to cellular stressors. Interestingly, the sensitivity to chemical stressors increased as

neural cells became more functionally similar to vulnerable cell types in PD, that is, iPSc-derived neural cells and neurons were more sensitive to mitochondrial stress than fibroblasts from the same patient. Recently, Sanders and colleagues report that iPSc-derived neural cells from subjects carrying G2019S or R1441C mutations in *LRRK2* exhibit elevated mitochondrial DNA (mtDNA) damage. These effects were specific to neural cell types and could be abolished by zinc finger nuclease-mediated correction of *LRRK2* genomic sequence (Sanders et al. 2014). Collectively, these observations connect *LRRK2* mutations with impaired mitochondrial function and indicate that *LRRK2* iPSc-derived neural cells are useful models for exploring neuronal vulnerability to stress and the identification of neuroprotective molecules. Studies of neural stem cells (NSCs) derived from G2019S PD subjects support a role for the nucleus as a cellular compartment implicated in PD (Liu et al. 2012). Liu et al. showed that G2019S *LRRK2* iPSc-derived NSCs display increased susceptibility to proteasomal stress, as well as passage-dependent deficiencies in nuclear envelope organization, clonal expansion, and neuronal differentiation. The passage-dependent alterations in nuclear morphology of G2019S NSCs correlated with epigenetic changes such as those observed during cellular aging and could potentially be mediated through phosphorylation of B-type lamins. Additionally, late-passage G2019S NSCs exhibited increased phosphorylation of LRRK2 and its putative substrate 4E-BP1. Pharmacological inhibition of LRRK2 kinase activity rescued the aberrant nuclear morphology and clonogenic capacity in G2019S iPSc-derived NSCs and restored a gene expression signature similar to WT *LRRK2* NSCs. In contrast to prior studies (Cooper et al. 2012), no evidence of altered mitochondria morphology was observed, which could result from differences in cellular differentiation protocols and/or the neural cell-type studied. Analysis of neuronal nuclear architecture in brains of idiopathic and G2019S *LRRK2* PD revealed that dentate gyrus neurons display altered nuclear morphology, whereas neurons of non-neurogenic areas do not exhibit alterations in nuclear envelope organization (Liu et al. 2012). These findings suggest a role for LRRK2 in neural stem cells and neural progenitors in adult neurogenic niches.

A recent report describes multiple phenotypes for iPSc-derived dopaminergic neurons derived from G2019S *LRRK2* PD subjects, including reduced neurite outgrowth and increased sensitivity to rotenone and 6-OHDA toxicity, which could be ameliorated by inhibition of LRRK2 kinase activity or by direct gene correction of the G2019S mutation (Reinhardt et al. 2013). G2019S iPSc-derived dopaminergic neurons displayed increased tau and α-synuclein protein levels, but without evidence of increased α-synuclein transcription. This finding suggests that the G2019S mutation may impair the degradation of α-synuclein. A second study in iPS cells further suggests an inhibitory role for LRRK2 in the clearance of α-synuclein by chaperone-mediated autophagy (Orenstein et al. 2013). Gene correction of the G2019S mutation in iPS cells enabled the identification of differentially regulated genes in G2019S *LRRK2* iPSc-derived dopaminergic neurons (Reinhardt et al. 2013). Alterations in the ERK signaling pathway and the *CPNE8*, *ANXA1*, *MAP7*, *CADPS2*, *MAPT,* and *UHRF2* genes were suggested to play a role in G2019S LRRK2-induced dopaminergic neuronal toxicity.

Mitochondrial and lysosomal dysfunction have also been reported in dopaminergic neurons derived from G2019S *LRRK2* iPS cells, such as loss of mitochondrial membrane potential, increased mitochondrial reactive oxygen species (mitoROS), and lysosomal hyperactivity (Su and Qi 2013). These effects are mediated by dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission. Drp1 inhibition corrected mitochondrial and lysosomal dysfunction and increased neurite length in G2019S iPSc-derived dopaminergic neurons. Inhibiting fission or increasing fusion of mitochondria may offer one potential approach to rescue mitochondrial dysfunction and enhance dopaminergic neuronal survival in G2019S *LRRK2* PD patients.

Collectively, iPSc-derived neurons provide important cellular models for understanding *LRRK2*-associated PD. iPSc-derived dopaminergic neurons display reduced neurite length, accumulation of α-synuclein and tau, increased vulnerability to cellular stress, and impaired autophagy and mitochondrial function. Inhibition of LRRK2 kinase activity rescued many of the pathological properties of the G2019S mutation, thereby confirming prior studies in LRRK2 animal models (Lee et al. 2010a). Interestingly, pathological features of *LRRK2* iPSc first appeared in aged cultures, thus suggesting age as a critical factor in their development as in PD. Furthermore, iPSc-based models can be successfully employed to discover and validate novel molecular pathways, organelles, and cellular populations potentially involved in *LRRK2*-associated PD. LRRK2 is abundantly expressed throughout the human brain, but is poorly expressed in dopamine-containing neurons (Galter et al. 2006). Therefore, the effects of familial mutations in endogenous LRRK2 might be subtle and difficult to identify in iPSc-derived dopaminergic neurons. It would be of interest to investigate the pathological consequences of LRRK2 mutations in other neuronal populations with higher endogenous expression of *LRRK2*, such as the cerebral cortex, striatum, hippocampus, or cerebellum. A major limitation of iPS cells is the poor efficiency of differentiating stem cells into specific neuronal subtypes such as functional dopaminergic neurons. Drug screening and target validation would be difficult to implement due to the scarcity of differentiated dopaminergic neurons in iPSc-derived cultures. Research efforts continue to focus on identifying key factors involved in human neural differentiation, and on the improvement of methods to produce specific neuronal subtypes that are relevant to PD.

3 Conclusion

3.1 What Have we Learned from LRRK2 Animal Models?

Despite the broad range of species employed for creating LRRK2 models, we are still some way from understanding the biological and pathophysiological function of LRRK2. The inability to consistently reproduce key phenotypes across different LRRK2 models has impeded the identification of common cellular processes regulated by LRRK2. LRRK2 animal models have provided support for a key role of kinase and GTPase activities in mediating LRRK2-dependent neuronal damage, and

have identified an important regulatory role for LRRK2 in striatal dopaminergic neurotransmission. Pharmacological inhibition of LRRK2 kinase activity in fly and rodent models could successfully attenuate dopaminergic neuronal degeneration induced by G2019S LRRK2, thereby supporting LRRK2 kinase activity as a key therapeutic target for treating PD. Studies are now warranted in animal models using newly developed kinase inhibitors with improved selectivity and potency for LRRK2 (Choi et al. 2012; Reith et al. 2012; Sheng et al. 2012), and for the development and validation of compounds that target the GTPase domain (Tsika and Moore 2013). Existing LRRK2 animal models provide a suitable resource in which to identify and validate therapeutic agents that modify LRRK2 activity and function as potential treatments for *LRRK2*-associated and potentially idiopathic PD.

LRRK2 animal models collectively recapitulate many of the key clinical and neuropathological features of *LRRK2*-associated PD, including the degeneration of nigrostriatal dopaminergic neurons, cytopathology, tau accumulation and hyperphosphorylation, abnormal striatal dopaminergic neurotransmission, and motoric deficits. However, the caveat is that many of these phenotypes do not often occur together in the same animal model, are not entirely robust, or do not act upon the nigrostriatal dopaminergic pathway. Although there is evidence for a pathological interaction between α-synuclein and LRRK2, studies in animal models are conflicting and difficult to reconcile, and it remains unclear whether LRRK2 is critical for the development of α-synuclein pathology. Whether α-synuclein is oppositely required for mediating LRRK2-induced neuropathology has not yet been evaluated. Mechanistically, LRRK2 plays an important role in neurite outgrowth and remodeling, and in cellular pathways previously implicated in the pathophysiology of PD including the oxidative stress response, autophagy, mitochondrial function, cytoskeleton organization and vesicular protein sorting. Animal models have also identified an unexpected biological role for LRRK2 in kidney and lung homeostasis, and in adult neurogenesis, which could potentially contribute to non-motor symptoms in PD (i.e., anxiety and depression).

3.2 Is There an Optimal LRRK2 Animal Model?

It is worth noting that despite the multitude of LRRK2 models developed so far, a single model is not able to faithfully recapitulate all clinical and neuropathological features of *LRRK2*-associated PD. The incomplete penetrance of *LRRK2* mutations and the diversity of clinical symptoms and neuropathology in *LRRK2* PD subjects may indicate a potential contribution of genetic and/or environmental factors to precipitating LRRK2-dependent neurotoxicity. What criteria should therefore be used to define an ideal animal model of LRRK2 pathobiology? The answer is that no animal model is perfect, but that all current LRRK2 models offer something different and all have their often unique differences and utilities, as we have attempted to emphasize throughout. In attempting to identify therapeutic agents for treating PD, the focus naturally levitates towards preventing or attenuating

neurodegeneration, one of the defining hallmarks of PD. Animal models that display LRRK2-mediated neurodegeneration should be considered as the most adequate for this task, although alternative robust LRRK2-related phenotypes may also prove sufficient (i.e., motoric deficits, cytopathology, or abnormal dopaminergic neurotransmission). Model organisms such as worms and flies, certain transgenic rodent models, and rodent viral models show sustained dopaminergic neurodegeneration upon overexpression of human G2019S LRRK2. Fly and worm models provide important and rapid tools for identifying and dissecting the novel molecular mechanisms underlying LRRK2-mediated neurodegeneration, whereas it is extremely time-consuming and laborious to perform such studies in transgenic rodent models. It is possible to readily evaluate the contribution of genetic and environmental factors to LRRK2-induced neurotoxicity in worm and fly models. However, the absence of a true LRRK2 homolog in *Drosophila* and *C. elegans* and the lack of conservation in brain complexity, organization, and neuronal circuitry, represent major deficiencies for the pre-clinical evaluation of therapeutic agents. Rodent models of LRRK2 are therefore necessary to corroborate findings from simpler organisms. Alternatively, iPSc-derived neuronal models permit one to study the consequences of *LRRK2* mutations directly on the cellular physiology of disease-relevant human neurons. Despite the promise of patient-derived iPS cells, the technology is not yet sufficiently advanced for high-throughput drug screening efforts, but already enables important insight into the cellular pathobiology of *LRRK2* mutations. Finally, viral-mediated gene transfer rodent models for human LRRK2 provide a promising approach to modeling PD. Adenovirus-mediated *LRRK2* gene delivery causes progressive dopaminergic neuronal degeneration within an acceptably short time frame ($\sim$6 weeks) and can be used to rapidly validate neuroprotective therapeutic targets and chemical agents. Importantly, this viral model can be applied to other environmental and/or genetic models of PD, which will broaden our understanding of the pathological pathways associated with LRRK2 in PD. Collectively, *LRRK2* animal models provide an important resource for the elucidation of novel disease mechanisms, the identification and validation of therapeutic targets, and for the evaluation of disease-modifying therapeutic agents.

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References

Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, Wade-Martins R (2009) *LRRK2* regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. Hum Mol Genet 18:4022–4034

Andres-Mateos E, Mejias R, Sasaki M, Li X, Lin BM, Biskup S, Zhang L, Banerjee R, Thomas B, Yang L, Liu G, Beal MF, Huso DL, Dawson TM, Dawson VL (2009) Unexpected lack of hypersensitivity in *LRRK2* knock-out mice to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). J Neurosci 29:15846–15850

Bailey RM, Covy JP, Melrose HL, Rousseau L, Watkinson R, Knight J, Miles S, Farrer MJ, Dickson DW, Giasson BI, Lewis J (2013) *LRRK2* phosphorylates novel tau epitopes and promotes tauopathy. Acta Neuropathol 126:809–827

Baptista MA, Dave KD, Frasier MA, Sherer TB, Greeley M, Beck MJ, Varsho JS, Parker GA, Moore C, Churchill MJ, Meshul CK, Fiske BK (2013) Loss of leucine-rich repeat kinase 2 (*LRRK2*) in rats leads to progressive abnormal phenotypes in peripheral organs. PLoS ONE 8:e80705

Beilina A, Rudenko IN, Kaganovich A, Civiero L, Chau H, Kalia SK, Kalia LV, Lobbestael E, Chia R, Ndukwe K, Ding J, Nalls MA, Olszewski M, Hauser DN, Kumaran R, Lozano AM, Baekelandt V, Greene LE, Taymans JM, Greggio E, Cookson MR (2014) Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporadic and familial Parkinson disease. Proc Natl Acad Sci USA 111:2626–2631

Berger Z, Smith KA, Lavoie MJ (2010) Membrane localization of *LRRK2* is associated with increased formation of the highly active *LRRK2* dimer and changes in its phosphorylation. Biochemistry 49:5511–5523

Bichler Z, Lim HC, Zeng L, Tan EK (2013) Non-motor and motor features in *LRRK2* transgenic mice. PLoS ONE 8:e70249

Biosa A, Trancikova A, Civiero L, Glauser L, Bubacco L, Greggio E, Moore DJ (2013) GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated *LRRK2*. Hum Mol Genet 22:1140–1156

Biskup S, Moore DJ, Celsi F, Higashi S, West AB, Andrabi SA, Kurkinen K, Yu SW, Savitt JM, Waldvogel HJ, Faull RL, Emson PC, Torp R, Ottersen OP, Dawson TM, Dawson VL (2006) Localization of *LRRK2* to membranous and vesicular structures in mammalian brain. Ann Neurol 60:557–569

Biskup S, Moore DJ, Rea A, Lorenz-Deperieux B, Coombes CE, Dawson VL, Dawson TM, West AB (2007) Dynamic and redundant regulation of *LRRK2* and *LRRK1* expression. BMC Neurosci 8:102

Biskup S, West AB (2009) Zeroing in on *LRRK2*-linked pathogenic mechanisms in Parkinson's disease. Biochim Biophys Acta 7:625–633

Bonifati V (2014) Genetics of Parkinson's disease: state of the art, 2013. Parkinsonism Relat Disord 20(Suppl 1):S23–S28

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256–259

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415

Chartier-Harlin MC, Dachsel JC, Vilarino-Guell C, Lincoln SJ, Lepretre F, Hulihan MM, Kachergus J, Milnerwood AJ, Tapia L, Song MS, Le Rhun E, Mutez E, Larvor L, Duflot A, Vanbesien-Mailliot C, Kreisler A, Ross OA, Nishioka K, Soto-Ortolaza AI, Cobb SA, Melrose HL, Behrouz B, Keeling BH, Bacon JA, Hentati E, Williams L, Yanagiya A, Sonenberg N, Lockhart PJ, Zubair AC, Uitti RJ, Aasly JO, Krygowska-Wajs A, Opala G, Wszolek ZK, Frigerio R, Maraganore DM, Gosal D, Lynch T, Hutchinson M, Bentivoglio AR, Valente EM, Nichols WC, Pankratz N, Foroud T, Gibson RA, Hentati F, Dickson DW, Destee A, Farrer MJ (2011) Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet 89:398–406

Chen CY, Weng YH, Chien KY, Lin KJ, Yeh TH, Cheng YP, Lu CS, Wang HL (2012) (G2019S) *LRRK2* activates MKK4-JNK pathway and causes degeneration of SN dopaminergic neurons in a transgenic mouse model of PD. Cell Death Differ 19:1623–1633

Chen L, Feany MB (2005) Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci 8:657–663

Choi HG, Zhang J, Deng X, Hatcher JM, Patricelli MP, Zhao Z, Alessi DR, Gray NS (2012) Brain penetrant *LRRK2* inhibitor. ACS Med Chem Lett 3:658–662

Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, Guo M (2006) Drosophila *PINK1* is required for mitochondrial function and interacts genetically with parkin. Nature 441:1162–1166

Cookson MR (2010) The role of leucine-rich repeat kinase 2 (*LRRK2*) in Parkinson's disease. Nat Rev Neurosci 11:791–797

Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathearn KE, Liu F, Cao S, Caldwell KA, Caldwell GA, Marsischky G, Kolodner RD, Labaer J, Rochet JC, Bonini NM, Lindquist S (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313:324–328

Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T, Hargus G, Deleidi M, Lawson T, Bogetofte H, Perez-Torres E, Clark L, Moskowitz C, Mazzulli J, Chen L, Volpicelli-Daley L, Romero N, Jiang H, Uitti RJ, Huang Z, Opala G, Scarffe LA, Dawson VL, Klein C, Feng J, Ross OA, Trojanowski JQ, Lee VM, Marder K, Surmeier DJ, Wszolek ZK, Przedborski S, Krainc D, Dawson TM, Isacson O (2012) Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. Sci Transl Med 4:3003985

Crosiers D, Theuns J, Cras P, Van Broeckhoven C (2011) Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. J Chem Neuroanat 42:131–141

Daher JP, Pletnikova O, Biskup S, Musso A, Gellhaar S, Galter D, Troncoso JC, Lee MK, Dawson TM, Dawson VL, Moore DJ (2012) Neurodegenerative phenotypes in an A53T alpha-synuclein transgenic mouse model are independent of *LRRK2*. Hum Mol Genet 21:2420–2431

Daniels V, Vancraenenbroeck R, Law BM, Greggio E, Lobbestael E, Gao F, De Maeyer M, Cookson MR, Harvey K, Baekelandt V, Taymans JM (2011) Insight into the mode of action of the *LRRK2* Y1699C pathogenic mutant. J Neurochem 116:304–315

Di Fonzo A, Dekker MC, Montagna P, Baruzzi A, Yonova EH, Correia Guedes L, Szczerbinska A, Zhao T, Dubbel-Hulsman LO, Wouters CH, de Graaff E, Oyen WJ, Simons EJ, Breedveld GJ, Oostra BA, Horstink MW, Bonifati V (2009) FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. Neurology 72:240–245

Dodson MW, Zhang T, Jiang C, Chen S, Guo M (2012) Roles of the Drosophila *LRRK2* homolog in Rab7-dependent lysosomal positioning. Hum Mol Genet 21:1350–1363

Dranka BP, Gifford A, Ghosh A, Zielonka J, Joseph J, Kanthasamy AG, Kalyanaraman B (2013) Diapocynin prevents early Parkinson's disease symptoms in the leucine-rich repeat kinase 2 (*LRRK2*R(1)(4)(4)(1)G) transgenic mouse. Neurosci Lett 549:57–62

Dusonchet J, Kochubey O, Stafa K, Young SM Jr, Zufferey R, Moore DJ, Schneider BL, Aebischer P (2011) A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in *LRRK2*. J Neurosci 31:907–912

Edvardson S, Cinnamon Y, Ta-Shma A, Shaag A, Yim YI, Zenvirt S, Jalas C, Lesage S, Brice A, Taraboulos A, Kaestner KH, Greene LE, Elpeleg O (2012) A deleterious mutation in *DNAJC6* encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS ONE 7:1

Galter D, Westerlund M, Carmine A, Lindqvist E, Sydow O, Olson L (2006) *LRRK2* expression linked to dopamine-innervated areas. Ann Neurol 59:714–719

Gasser T (2009) Mendelian forms of Parkinson's disease. Biochim Biophys Acta 7:587–596

Gehrke S, Imai Y, Sokol N, Lu B (2010) Pathogenic *LRRK2* negatively regulates microRNA-mediated translational repression. Nature 466:637–641

Giasson BI, Covy JP, Bonini NM, Hurtig HI, Farrer MJ, Trojanowski JQ, Van Deerlin VM (2006) Biochemical and pathological characterization of *LRRK2*. Ann Neurol 59:315–322

Giesert F, Hofmann A, Burger A, Zerle J, Kloos K, Hafen U, Ernst L, Zhang J, Vogt-Weisenhorn DM, Wurst W (2013) Expression analysis of *LRRK1*, *LRRK2* and *LRRK2* splice variants in mice. PLoS One 8

Gillardon F (2009) Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability—a point of convergence in parkinsonian neurodegeneration? J Neurochem 110:1514–1522

Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Rochet JC, McCaffery JM, Barlowe C, Lindquist S (2008) The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. Proc Natl Acad Sci USA 105:145–150

Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S (2009) Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat Genet 41:308–315

Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, Kolch W, Prokisch H, Ueffing M (2006) The Parkinson disease causing *LRRK2* mutation I2020T is associated with increased kinase activity. Hum Mol Genet 15:223–232

Gloeckner CJ, Schumacher A, Boldt K, Ueffing M (2009) The Parkinson disease-associated protein kinase *LRRK2* exhibits MAPKKK activity and phosphorylates MKK3/6 and MKK4/7, in vitro. J Neurochem 109:959–968

Greggio E, Cookson MR (2009) Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN Neuro 1

Greggio E, Taymans JM, Zhen EY, Ryder J, Vancraenenbroeck R, Beilina A, Sun P, Deng J, Jaffe H, Baekelandt V, Merchant K, Cookson MR (2009) The Parkinson's disease kinase *LRRK2* autophosphorylates its GTPase domain at multiple sites. Biochem Biophys Res Commun 389:449–454

Greggio E, Zambrano I, Kaganovich A, Beilina A, Taymans JM, Daniels V, Lewis P, Jain S, Ding J, Syed A, Thomas KJ, Baekelandt V, Cookson MR (2008) The Parkinson disease-associated leucine-rich repeat kinase 2 (*LRRK2*) is a dimer that undergoes intramolecular autophosphorylation. J Biol Chem 283:16906–16914

Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbach G, Venderova K, Girardin SE, Bulman DE, Scherzer CR, LaVoie MJ, Gris D, Park DS, Angel JB, Shen J, Philpott DJ, Schlossmacher MG (2011) Parkinson's disease-linked *LRRK2* is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. J Neural Transm 118:795–808

Hatano T, Kubo S, Imai S, Maeda M, Ishikawa K, Mizuno Y, Hattori N (2007) Leucine-rich repeat kinase 2 associates with lipid rafts. Hum Mol Genet 16:678–690

Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, Brice A, Aasly J, Zabetian CP, Goldwurm S, Ferreira JJ, Tolosa E, Kay DM, Klein C, Williams DR, Marras C, Lang AE, Wszolek ZK, Berciano J, Schapira AH, Lynch T, Bhatia KP, Gasser T, Lees AJ, Wood NW (2008) Phenotype, genotype, and worldwide genetic penetrance of *LRRK2*-associated Parkinson's disease: a case-control study. Lancet Neurol 7:583–590

Herzig MC, Bidinosti M, Schweizer T, Hafner T, Stemmelen C, Weiss A, Danner S, Vidotto N, Stauffer D, Barske C, Mayer F, Schmid P, Rovelli G, van der Putten PH, Shimshek DR (2012) High *LRRK2* levels fail to induce or exacerbate neuronal alpha-synucleinopathy in mouse brain. PLoS ONE 7:15

Herzig MC, Kolly C, Persohn E, Theil D, Schweizer T, Hafner T, Stemmelen C, Troxler TJ, Schmid P, Danner S, Schnell CR, Mueller M, Kinzel B, Grevot A, Bolognani F, Stirn M, Kuhn RR, Kaupmann K, van der Putten PH, Rovelli G, Shimshek DR (2011) *LRRK2* protein levels are determined by kinase function and are crucial for kidney and lung homeostasis in mice. Hum Mol Genet 20:4209–4223

Hindle S, Afsari F, Stark M, Middleton CA, Evans GJ, Sweeney ST, Elliott CJ (2013) Dopaminergic expression of the Parkinsonian gene *LRRK2*-G2019S leads to non-autonomous visual neurodegeneration, accelerated by increased neural demands for energy. Hum Mol Genet 22:2129–2140

Hinkle KM, Yue M, Behrouz B, Dachsel JC, Lincoln SJ, Bowles EE, Beevers JE, Dugger B, Winner B, Prots I, Kent CB, Nishioka K, Lin WL, Dickson DW, Janus CJ, Farrer MJ, Melrose HL (2012) *LRRK2* knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. Mol Neurodegener 7:25

Hulihan MM, Ishihara-Paul L, Kachergus J, Warren L, Amouri R, Elango R, Prinjha RK, Upmanyu R, Kefi M, Zouari M, Sassi SB, Yahmed SB, El Euch-Fayeche G, Matthews PM, Middleton LT, Gibson RA, Hentati F, Farrer MJ (2008) *LRRK2* Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. Lancet Neurol 7:591–594

Imai Y, Gehrke S, Wang HQ, Takahashi R, Hasegawa K, Oota E, Lu B (2008) Phosphorylation of 4E-BP by *LRRK2* affects the maintenance of dopaminergic neurons in Drosophila. EMBO J 27:2432–2443

International Parkinson Disease Genomics C, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, Saad M, Simon-Sanchez J, Schulte C, Lesage S, Sveinbjornsdottir S, Stefansson K, Martinez M, Hardy J, Heutink P, Brice A, Gasser T, Singleton AB, Wood NW (2011) Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 377:641–649

Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, Iwatsubo T (2007) GTP binding is essential to the protein kinase activity of *LRRK2*, a causative gene product for familial Parkinson's disease. Biochemistry 46:1380–1388

Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, Alessi DR (2007) *LRRK2* phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. Biochem J 405:307–317

James NG, Digman MA, Gratton E, Barylko B, Ding X, Albanesi JP, Goldberg MS, Jameson DM (2012) Number and brightness analysis of *LRRK2* oligomerization in live cells. Biophys J 102:L41–L43

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–376

Kamikawaji S, Ito G, Sano T, Iwatsubo T (2013) Differential effects of familial Parkinson mutations in *LRRK2* Revealed by a systematic analysis of autophosphorylation. Biochemistry 23:23

Kanao T, Venderova K, Park DS, Unterman T, Lu B, Imai Y (2010) Activation of FoxO by *LRRK2* induces expression of proapoptotic proteins and alters survival of postmitotic dopaminergic neuron in Drosophila. Hum Mol Genet 19:3747–3758

Kawakami F, Yabata T, Ohta E, Maekawa T, Shimada N, Suzuki M, Maruyama H, Ichikawa T, Obata F (2012) *LRRK2* phosphorylates tubulin-associated tau but not the free molecule: *LRRK2*-mediated regulation of the tau-tubulin association and neurite outgrowth. PLoS ONE 7:27

Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ, Bjorklund A (2002) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J Neurosci 22:2780–2791

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392:605–608

Klein RL, King MA, Hamby ME, Meyer EM (2002) Dopaminergic cell loss induced by human A30P alpha-synuclein gene transfer to the rat substantia nigra. Hum Gene Ther 13:605–612

Krebs CE, Karkheiran S, Powell JC, Cao M, Makarov V, Darvish H, Di Paolo G, Walker RH, Shahidi GA, Buxbaum JD, De Camilli P, Yue Z, Paisan-Ruiz C (2013) The Sac1 domain of *SYNJ1* identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. Hum Mutat 34:1200–1207

Lang AE, Lozano AM (1998a) Parkinson's disease. First of two parts. N Engl J Med 339:1044–1053

Lang AE, Lozano AM (1998b) Parkinson's disease. Second of two parts. N Engl J Med 339:1130–1143

Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, Lee YI, Maguire-Zeiss KA, Bowers WJ, Federoff HJ, Dawson VL, Dawson TM (2010a) Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. Nat Med 16:998–1000

Lee S, Liu HP, Lin WY, Guo H, Lu B (2010b) *LRRK2* kinase regulates synaptic morphology through distinct substrates at the presynaptic and postsynaptic compartments of the Drosophila neuromuscular junction. J Neurosci 30:16959–16969

Lee SB, Kim W, Lee S, Chung J (2007) Loss of *LRRK2*/PARK8 induces degeneration of dopaminergic neurons in Drosophila. Biochem Biophys Res Commun 358:534–539

Lewis PA, Greggio E, Beilina A, Jain S, Baker A, Cookson MR (2007) The R1441C mutation of *LRRK2* disrupts GTP hydrolysis. Biochem Biophys Res Commun 357:668–671

Li X, Patel JC, Wang J, Avshalumov MV, Nicholson C, Buxbaum JD, Elder GA, Rice ME, Yue Z (2010) Enhanced striatal dopamine transmission and motor performance with *LRRK2* overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. J Neurosci 30:1788–1797

Li X, Tan YC, Poulose S, Olanow CW, Huang XY, Yue Z (2007) Leucine-rich repeat kinase 2 (*LRRK2*)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants. J Neurochem 103:238–247

Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C (2009) Mutant *LRRK2*(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci 12:826–828

Liao J, Wu CX, Burlak C, Zhang S, Sahm H, Wang M, Zhang ZY, Vogel KW, Federici M, Riddle SM, Nichols RJ, Liu D, Cookson MR, Stone TA, Hoang QQ (2014) Parkinson disease-associated mutation R1441H in *LRRK2* prolongs the "active state" of its GTPase domain. Proc Natl Acad Sci USA 3:3

Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, Schjeide LM, Meissner E, Zauft U, Allen NC, Liu T, Schilling M, Anderson KJ, Beecham G, Berg D, Biernacka JM, Brice A, DeStefano AL, Do CB, Eriksson N, Factor SA, Farrer MJ, Foroud T, Gasser T, Hamza T, Hardy JA, Heutink P, Hill-Burns EM, Klein C, Latourelle JC, Maraganore DM, Martin ER, Martinez M, Myers RH, Nalls MA, Pankratz N, Payami H, Satake W, Scott WK, Sharma M, Singleton AB, Stefansson K, Toda T, Tung JY, Vance J, Wood NW, Zabetian CP, Young P, Tanzi RE, Khoury MJ, Zipp F, Lehrach H, Ioannidis JP, Bertram L (2012) Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: the PDGene database. PLoS Genet 8:15

Lin CH, Tsai PI, Wu RM, Chien CT (2010) *LRRK2* G2019S mutation induces dendrite degeneration through mislocalization and phosphorylation of tau by recruiting autoactivated GSK3ss. J Neurosci 30:13138–13149

Lin X, Parisiadou L, Gu XL, Wang L, Shim H, Sun L, Xie C, Long CX, Yang WJ, Ding J, Chen ZZ, Gallant PE, Tao-Cheng JH, Rudow G, Troncoso JC, Liu Z, Li Z, Cai H (2009) Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. Neuron 64:807–827

Liu HF, Lu S, Ho PWL, Tse HM, Pang SYY, Kung MHW, Ho JWM, Ramsden DB, Zhou ZJ, Ho SL (2014) *LRRK2* R1441G mice are more liable to dopamine depletion and locomotor inactivity. Ann Clin Trans Neurol 1:199–208

Liu GH, Qu J, Suzuki K, Nivet E, Li M, Montserrat N, Yi F, Xu X, Ruiz S, Zhang W, Wagner U, Kim A, Ren B, Li Y, Goebl A, Kim J, Soligalla RD, Dubova I, Thompson J, Yates J 3rd, Esteban CR, Sancho-Martinez I, Izpisua Belmonte JC (2012) Progressive degeneration of human neural stem cells caused by pathogenic *LRRK2*. Nature 491:603–607

Liu Z, Hamamichi S, Lee BD, Yang D, Ray A, Caldwell GA, Caldwell KA, Dawson TM, Smith WW, Dawson VL (2011) Inhibitors of *LRRK2* kinase attenuate neurodegeneration and Parkinson-like phenotypes in Caenorhabditis elegans and Drosophila Parkinson's disease models. Hum Mol Genet 20:3933–3942

Liu Z, Wang X, Yu Y, Li X, Wang T, Jiang H, Ren Q, Jiao Y, Sawa A, Moran T, Ross CA, Montell C, Smith WW (2008) A Drosophila model for *LRRK2*-linked Parkinsonism. Proc Natl Acad Sci USA 105:2693–2698

Lo Bianco C, Ridet JL, Schneider BL, Deglon N, Aebischer P (2002) Alpha-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci USA 99:10813–10818

Low K, Aebischer P (2012) Use of viral vectors to create animal models for Parkinson's disease. Neurobiol Dis 48:189–201

MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A (2006) The familial Parkinsonism gene *LRRK2* regulates neurite process morphology. Neuron 52:587–593

MacLeod DA, Rhinn H, Kuwahara T, Zolin A, Di Paolo G, McCabe BD, Marder KS, Honig LS, Clark LN, Small SA, Abeliovich A (2013) RAB7L1 interacts with *LRRK2* to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron 77:425–439

Mandemakers W, Snellinx A, O'Neill MJ, de Strooper B (2012) *LRRK2* expression is enriched in the striosomal compartment of mouse striatum. Neurobiol Dis 48:582–593

Marin I (2008) Ancient origin of the Parkinson disease gene *LRRK2*. J Mol Evol 67:41–50

Matta S, Van Kolen K, da Cunha R, van den Bogaart G, Mandemakers W, Miskiewicz K, De Bock PJ, Morais VA, Vilain S, Haddad D, Delbroek L, Swerts J, Chavez-Gutierrez L, Esposito G, Daneels G, Karran E, Holt M, Gevaert K, Moechars DW, De Strooper B, Verstreken P (2012) *LRRK2* controls an EndoA phosphorylation cycle in synaptic endocytosis. Neuron 75:1008–1021

Melrose HL, Dachsel JC, Behrouz B, Lincoln SJ, Yue M, Hinkle KM, Kent CB, Korvatska E, Taylor JP, Witten L, Liang YQ, Beevers JE, Boules M, Dugger BN, Serna VA, Gaukhman A, Yu X, Castanedes-Casey M, Braithwaite AT, Ogholikhan S, Yu N, Bass D, Tyndall G, Schellenberg GD, Dickson DW, Janus C, Farrer MJ (2010) Impaired dopaminergic neurotransmission and microtubule-associated protein tau alterations in human *LRRK2* transgenic mice. Neurobiol Dis 40:503–517

Meulener M, Whitworth AJ, Armstrong-Gold CE, Rizzu P, Heutink P, Wes PD, Pallanck LJ, Bonini NM (2005) Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. Curr Biol 15:1572–1577

Moehle MS, Webber PJ, Tse T, Sukar N, Standaert DG, DeSilva TM, Cowell RM, West AB (2012) *LRRK2* inhibition attenuates microglial inflammatory responses. J Neurosci 32:1602–1611 The Official Journal of the Society for Neuroscience

Ness D, Ren Z, Gardai S, Sharpnack D, Johnson VJ, Brennan RJ, Brigham EF, Olaharski AJ (2013) Leucine-rich repeat kinase 2 (*LRRK2*)-deficient rats exhibit renal tubule injury and perturbations in metabolic and immunological homeostasis. PLoS One 8

Ng CH, Guan MS, Koh C, Ouyang X, Yu F, Tan EK, O'Neill SP, Zhang X, Chung J, Lim KL (2012) AMP kinase activation mitigates dopaminergic dysfunction and mitochondrial abnormalities in Drosophila models of Parkinson's disease. J Neurosci 32:14311–14317

Ng CH, Mok SZ, Koh C, Ouyang X, Fivaz ML, Tan EK, Dawson VL, Dawson TM, Yu F, Lim KL (2009) Parkin protects against *LRRK2* G2019S mutant-induced dopaminergic neurodegeneration in Drosophila. J Neurosci 29:11257–11262

Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schule B, Dolmetsch RE, Langston W, Palmer TD, Pera RR (2011) *LRRK2* mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 8:267–280

Ohta E, Kawakami F, Kubo M, Obata F (2011) *LRRK2* directly phosphorylates Akt1 as a possible physiological substrate: impairment of the kinase activity by Parkinson's disease-associated mutations. FEBS Lett 585:2165–2170

Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, Cortes E, Honig LS, Dauer W, Consiglio A, Raya A, Sulzer D, Cuervo AM (2013) Interplay of *LRRK2* with chaperone-mediated autophagy. Nat Neurosci 16:394–406

Outeiro TF, Lindquist S (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302:1772–1775

Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB (2004) Cloning of the gene containing mutations that cause *PARK8*-linked Parkinson's disease. Neuron 44:595–600

Parisiadou L, Xie C, Cho HJ, Lin X, Gu XL, Long CX, Lobbestael E, Baekelandt V, Taymans JM, Sun L, Cai H (2009) Phosphorylation of ezrin/radixin/moesin proteins by *LRRK2* promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. J Neurosci 29:13971–13980

Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ (2008) Disease-specific induced pluripotent stem cells. Cell 134:877–886

Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J (2006) Mitochondrial dysfunction in Drosophila *PINK1* mutants is complemented by parkin. Nature 441:1157–1161

Paus M, Kohl Z, Ben Abdallah NM, Galter D, Gillardon F, Winkler J (2013) Enhanced dendritogenesis and axogenesis in hippocampal neuroblasts of *LRRK2* knockout mice. Brain Res 25:85–100

Pereira C, Miguel Martins L, Saraiva L (2014) *LRRK2*, but not pathogenic mutants, protects against HO stress depending on mitochondrial function and endocytosis in a yeast model. Biochim Biophys Acta 24:00072–00076

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047

Qing H, Wong W, McGeer EG, McGeer PL (2009) *LRRK2* phosphorylates alpha synuclein at serine 129: Parkinson disease implications. Biochem Biophys Res Commun 387:149–152

Quadri M, Fang M, Picillo M, Olgiati S, Breedveld GJ, Graafland J, Wu B, Xu F, Erro R, Amboni M, Pappata S, Quarantelli M, Annesi G, Quattrone A, Chien HF, Barbosa ER, Oostra BA, Barone P, Wang J, Bonifati V (2013) Mutation in the *SYNJ1* gene associated with autosomal recessive, early-onset Parkinsonism. Hum Mutat 34:1208–1215

Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, Al-Din A, Hillmer AM, Karsak M, Liss B, Woods CG, Behrens MI, Kubisch C (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 38:1184–1191

Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, Dawson TM, Dawson VL, Moore DJ (2011) Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant *LRRK2*. PLoS ONE 6:0018568

Reinhardt P, Schmid B, Burbulla LF, Schondorf DC, Wagner L, Glatza M, Hoing S, Hargus G, Heck SA, Dhingra A, Wu G, Muller S, Brockmann K, Kluba T, Maisel M, Kruger R, Berg D, Tsytsyura Y, Thiel CS, Psathaki OE, Klingauf J, Kuhlmann T, Klewin M, Muller H, Gasser T, Scholer HR, Sterneckert J (2013) Genetic correction of a *LRRK2* mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. Cell Stem Cell 12:354–367

Reith AD, Bamborough P, Jandu K, Andreotti D, Mensah L, Dossang P, Choi HG, Deng X, Zhang J, Alessi DR, Gray NS (2012) GSK2578215A; a potent and highly selective 2-arylmethyloxy-5-substitutent-N-arylbenzamide *LRRK2* kinase inhibitor. Bioorg Med Chem Lett 22:5625–5629

Ren G, Xin S, Li S, Zhong H, Lin S (2011) Disruption of *LRRK2* does not cause specific loss of dopaminergic neurons in zebrafish. PLoS ONE 6:16

Ross OA, Toft M, Whittle AJ, Johnson JL, Papapetropoulos S, Mash DC, Litvan I, Gordon MF, Wszolek ZK, Farrer MJ, Dickson DW (2006) *LRRK2* and Lewy body disease. Ann Neurol 59:388–393

Saha S, Guillily MD, Ferree A, Lanceta J, Chan D, Ghosh J, Hsu CH, Segal L, Raghavan K, Matsumoto K, Hisamoto N, Kuwahara T, Iwatsubo T, Moore L, Goldstein L, Cookson M, Wolozin B (2009) *LRRK2* modulates vulnerability to mitochondrial dysfunction in *Caenorhabditis elegans*. J Neurosci 29:9210–9218

Sakaguchi-Nakashima A, Meir JY, Jin Y, Matsumoto K, Hisamoto N (2007) *LRK-1*, a C. elegans PARK8-related kinase, regulates axonal-dendritic polarity of SV proteins. Curr Biol 17:592–598

Samann J, Hegermann J, von Gromoff E, Eimer S, Baumeister R, Schmidt E (2009) *Caenorhabditits elegans LRK-1* and *PINK1* act antagonistically in stress response and neurite outgrowth. J Biol Chem 284:16482–16491

Sanchez-Danes A, Richaud-Patin Y, Carballo-Carbajal I, Jimenez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giralt A, Canals JM, Memo M, Alberch J, Lopez-Barneo J, Vila M, Cuervo AM, Tolosa E, Consiglio A, Raya A (2012) Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. EMBO Mol Med 4:380–395

Sanders LH, Laganiere J, Cooper O, Mak SK, Vu BJ, Huang YA, Paschon DE, Vangipuram M, Sundararajan R, Urnov FD, Langston JW, Gregory PD, Zhang HS, Greenamyre JT, Isacson O, Schule B (2014) *LRRK2* mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. Neurobiol Dis 62:381–386

Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 41:1303–1307

Sen S, Webber PJ, West AB (2009) Dependence of leucine-rich repeat kinase 2 (*LRRK2*) kinase activity on dimerization. J Biol Chem 284:36346–36356

Sheng D, Qu D, Kwok KH, Ng SS, Lim AY, Aw SS, Lee CW, Sung WK, Tan EK, Lufkin T, Jesuthasan S, Sinnakaruppan M, Liu J (2010) Deletion of the WD40 domain of *LRRK2* in Zebrafish causes Parkinsonism-like loss of neurons and locomotive defect. PLoS Genet 6:1000914

Sheng Z, Zhang S, Bustos D, Kleinheinz T, Le Pichon CE, Dominguez SL, Solanoy HO, Drummond J, Zhang X, Ding X, Cai F, Song Q, Li X, Yue Z, van der Brug MP, Burdick DJ, Gunzner-Toste J, Chen H, Liu X, Estrada AA, Sweeney ZK, Scearce-Levie K, Moffat JG, Kirkpatrick DS, Zhu H (2012) Ser1292 autophosphorylation is an indicator of *LRRK2* kinase activity and contributes to the cellular effects of PD mutations. Sci Transl Med 4:3004485

Shiba Y, Randazzo PA (2012) ArfGAP1 function in COPI mediated membrane traffic: currently debated models and comparison to other coat-binding ArfGAPs. Histol Histopathol 27:1143–1153

Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41:1308–1312

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) Alpha-Synuclein locus triplication causes Parkinson's disease. Science 302:841

Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136:964–977

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840

Stafa K, Trancikova A, Webber PJ, Glauser L, West AB, Moore DJ (2012) GTPase activity and neuronal toxicity of Parkinson's disease-associated *LRRK2* is regulated by ArfGAP1. PLoS Genet 8:9

Su YC, Qi X (2013) Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by *LRRK2* G2019S mutation. Hum Mol Genet 27:27

Tain LS, Mortiboys H, Tao RN, Ziviani E, Bandmann O, Whitworth AJ (2009) Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. Nat Neurosci 12:1129–1135

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872

Taymans JM, Vancraenenbroeck R, Ollikainen P, Beilina A, Lobbestael E, De Maeyer M, Baekelandt V, Cookson MR (2011) *LRRK2* kinase activity is dependent on *LRRK2* GTP binding capacity but independent of *LRRK2* GTP binding. PLoS ONE 6:e23207

Tong Y, Pisani A, Martella G, Karouani M, Yamaguchi H, Pothos EN, Shen J (2009) R1441C mutation in *LRRK2* impairs dopaminergic neurotransmission in mice. Proc Natl Acad Sci USA 106:14622–14627

Tong Y, Yamaguchi H, Giaime E, Boyle S, Kopan R, Kelleher RJ 3rd, Shen J (2010) Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. Proc Natl Acad Sci USA 107:9879–9884

Trancikova A, Mamais A, Webber PJ, Stafa K, Tsika E, Glauser L, West AB, Bandopadhyay R, Moore DJ (2012) Phosphorylation of 4E-BP1 in the mammalian brain is not altered by *LRRK2* expression or pathogenic mutations. PLoS ONE 7:e47784

Tsika E, Moore DJ (2012) Mechanisms of *LRRK2*-mediated neurodegeneration. Curr Neurol Neurosci Rep 12:251–260

Tsika E, Moore DJ (2013) Contribution of GTPase activity to *LRRK2*-associated Parkinson disease. Small GTPases 4

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW (2004) Hereditary early-onset Parkinson's disease caused by mutations in *PINK1*. Science 304:1158–1160

Venderova K, Kabbach G, Abdel-Messih E, Zhang Y, Parks RJ, Imai Y, Gehrke S, Ngsee J, Lavoie MJ, Slack RS, Rao Y, Zhang Z, Lu B, Haque ME, Park DS (2009) Leucine-rich repeat kinase 2 interacts with Parkin, DJ-1 and *PINK1* in a *Drosophila melanogaster* model of Parkinson's disease. Hum Mol Genet 18:4390–4404

Vilarino-Guell C, Wider C, Ross OA, Dachsel JC, Kachergus JM, Lincoln SJ, Soto-Ortolaza AI, Cobb SA, Wilhoite GJ, Bacon JA, Behrouz B, Melrose HL, Hentati E, Puschmann A, Evans DM, Conibear E, Wasserman WW, Aasly JO, Burkhard PR, Djaldetti R, Ghika J, Hentati F, Krygowska-Wajs A, Lynch T, Melamed E, Rajput A, Rajput AH, Solida A, Wu RM, Uitti RJ, Wszolek ZK, Vingerhoets F, Farrer MJ (2011) *VPS35* mutations in Parkinson disease. Am J Hum Genet 89:162–167

Wang D, Tang B, Zhao G, Pan Q, Xia K, Bodmer R, Zhang Z (2008a) Dispensable role of Drosophila ortholog of *LRRK2* kinase activity in survival of dopaminergic neurons. Mol Neurodegener 3:3

Wang L, Xie C, Greggio E, Parisiadou L, Shim H, Sun L, Chandran J, Lin X, Lai C, Yang WJ, Moore DJ, Dawson TM, Dawson VL, Chiosis G, Cookson MR, Cai H (2008b) The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. J Neurosci 28:3384–3391

Webber PJ, Smith AD, Sen S, Renfrow MB, Mobley JA, West AB (2011) Autophosphorylation in the leucine-rich repeat kinase 2 (*LRRK2*) GTPase domain modifies kinase and GTP-binding activities. J Mol Biol 412:94–110

West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, Dawson VL, Dawson TM (2005) Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci USA 102:16842–16847

West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, Zhang Z, Lim KL, Dawson VL, Dawson TM (2007) Parkinson's disease-associated mutations in *LRRK2* link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet 16:223–232

Westerlund M, Belin AC, Anvret A, Bickford P, Olson L, Galter D (2008) Developmental regulation of leucine-rich repeat kinase 1 and 2 expression in the brain and other rodent and human organs: implications for Parkinson's disease. Neuroscience 152:429–436

Willingham S, Outeiro TF, DeVit MJ, Lindquist SL, Muchowski PJ (2003) Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. Science 302:1769–1772

Winner B, Melrose HL, Zhao C, Hinkle KM, Yue M, Kent C, Braithwaite AT, Ogholikhan S, Aigner R, Winkler J, Farrer MJ, Gage FH (2011) Adult neurogenesis and neurite outgrowth are impaired in *LRRK2* G2019S mice. Neurobiol Dis 41:706–716

Wolozin B, Saha S, Guillily M, Ferree A, Riley M (2008) Investigating convergent actions of genes linked to familial Parkinson's disease. Neurodegener Dis 5:182–185

Xiong Y, Coombes CE, Kilaru A, Li X, Gitler AD, Bowers WJ, Dawson VL, Dawson TM, Moore DJ (2010) GTPase activity plays a key role in the pathobiology of *LRRK2*. PLoS Genet 6:1000902

Xiong Y, Yuan C, Chen R, Dawson TM, Dawson VL (2012) ArfGAP1 is a GTPase activating protein for *LRRK2*: reciprocal regulation of ArfGAP1 by *LRRK2*. J Neurosci 32:3877–3886

Yao C, El Khoury R, Wang W, Byrd TA, Pehek EA, Thacker C, Zhu X, Smith MA, Wilson-Delfosse AL, Chen SG (2010) *LRRK2*-mediated neurodegeneration and dysfunction of dopaminergic neurons in a *Caenorhabditis elegans* model of Parkinson's disease. Neurobiol Dis 40:73–81

Yao C, Johnson WM, Gao Y, Wang W, Zhang J, Deak M, Alessi DR, Zhu X, Mieyal JJ, Roder H, Wilson-Delfosse AL, Chen SG (2013) Kinase inhibitors arrest neurodegeneration in cell and *C. elegans* models of *LRRK2* toxicity. Hum Mol Genet 22:328–344

Yeger-Lotem E, Riva L, Su LJ, Gitler AD, Cashikar AG, King OD, Auluck PK, Geddie ML, Valastyan JS, Karger DR, Lindquist S, Fraenkel E (2009) Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. Nat Genet 41:316–323

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920

Yuan Y, Cao P, Smith MA, Kramp K, Huang Y, Hisamoto N, Matsumoto K, Hatzoglou M, Jin H, Feng Z (2011) Dysregulated *LRRK2* signaling in response to endoplasmic reticulum stress leads to dopaminergic neuron degeneration in *C. elegans*. PLoS ONE 6:3

Yun HJ, Park J, Ho DH, Kim H, Kim CH, Oh H, Ga I, Seo H, Chang S, Son I, Seol W (2013) *LRRK2* phosphorylates Snapin and inhibits interaction of Snapin with SNAP-25. Exp Mol Med 16:68

Zhou H, Huang C, Tong J, Hong WC, Liu YJ, Xia XG (2011) Temporal expression of mutant *LRRK2* in adult rats impairs dopamine reuptake. Int J Biol Sci 7:753–761

Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, Haubenberger D, Spielberger S, Schulte EC, Lichtner P, Rossle SC, Klopp N, Wolf E, Seppi K, Pirker W, Presslauer S, Mollenhauer B, Katzenschlager R, Foki T, Hotzy C, Reinthaler E, Harutyunyan A, Kralovics R, Peters A, Zimprich F, Brucke T, Poewe W, Auff E, Trenkwalder C, Rost B, Ransmayr G, Winkelmann J, Meitinger T, Strom TM (2011) A mutation in *VPS35*, encoding a subunit of the retromer complex, causes late-onset Parkinson's disease. Am J Hum Genet 89:168–175

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T (2004) Mutations in *LRRK2* cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44:601–607

Models of Multiple System Atrophy

Lisa Fellner, Gregor K. Wenning and Nadia Stefanova

Abstract Multiple system atrophy (MSA) is a predominantly sporadic, adult-onset, fatal neurodegenerative disease of unknown etiology. MSA is characterized by autonomic failure, levodopa-unresponsive parkinsonism, cerebellar ataxia and pyramidal signs in any combination. MSA belongs to a group of neurodegenerative disorders termed α-synucleinopathies, which also include Parkinson's disease and dementia with Lewy bodies. Their common pathological feature is the occurrence of abnormal α-synuclein positive inclusions in neurons or glial cells. In MSA, the main cell type presenting aggregates composed of α-synuclein are oligodendroglial cells. This pathological hallmark, also called glial cytoplasmic inclusions (GCIs), is associated with progressive and profound neuronal loss in various regions of the brain. The development of animal models of MSA is justified by the limited understanding of the mechanisms of neurodegeneration and GCIs formation, which is paralleled by a lack of therapeutic strategies. Two main types of rodent models have been generated to replicate different features of MSA neuropathology. On one hand, neurotoxin-based models have been produced to reproduce neuronal loss in substantia nigra pars compacta and striatum. On the other hand, transgenic mouse models with overexpression of α-synuclein in oligodendroglia have been used to reproduce GCIs-related pathology. This chapter gives an overview of the atypical Parkinson's syndrome MSA and summarizes the currently available MSA animal models and their relevance for pre-clinical testing of disease-modifying therapies.

L. Fellner (✉) · G. K. Wenning · N. Stefanova
Division of Neurobiology, Department of Neurology, Innsbruck Medical University,
Anichstrasse 35, 6020 Innsbruck, Austria
e-mail: lisa.fellner@i-med.ac.at

G. K. Wenning
e-mail: gregor.wenning@i-med.ac.at

N. Stefanova
e-mail: nadia.stefanova@i-med.ac.at

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1 Introduction to MSA

Graham and Oppenheimer first introduced the term multiple system atrophy (MSA) in 1969 (Graham and Oppenheimer 1969) to combine different clinico-pathological disorders, including olivopontocerebellar atrophy (Déjérine and Thomas 1900), Shy Drager syndrome (Shy and Drager 1960), and striatonigral degeneration (SND) (Adams et al. 1964). MSA is now defined as a progressive neurodegenerative disorder which presents with autonomic failure, cerebellar ataxia, pyramidal signs, and parkinsonism in any combination (Wenning et al. 2004a). Due to the levodopa refractory Parkinsonism that is associated with different distinctive atypical features, MSA is categorized among atypical parkinsonian disorders (APD), including dementia with Lewy bodies (DLB), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) (Wenning et al. 2011a, b). Disease onset is usually in the sixth decade with an annual worldwide incidence rate below 1 in 100,000. However, disease incidence increases to 3/100,000 in the population over 50 years (Schrag et al. 1999; Vanacore et al. 2001; Stefanova et al. 2009a). MSA patients have a poor prognosis compared to Parkinson's disease (PD) patients. The mean survival rate ranges between 7 and 9 years following initial clinical presentation (Schrag et al. 2008). An early presentation of autonomic failure, as well as female gender, the parkinsonian variant of MSA, older age of onset, and shorter interval to reach clinical milestones (e.g., frequent falling, dysphagia, wheelchair dependency) predict shortened survival (Tada et al. 2007; O'Sullivan et al. 2008; Wenning et al. 2013).

MSA is clinically divided into a parkinsonian type (MSA-P) or a cerebellar type (MSA-C), which respectively relates to damage of either the basal ganglia (striatonigral degeneration, SND) or cerebellum (olivopontocerebellar atrophy, OPCA) (Ubhi et al. 2011). Parkinsonism is defined by bradykinesia, postural instability,

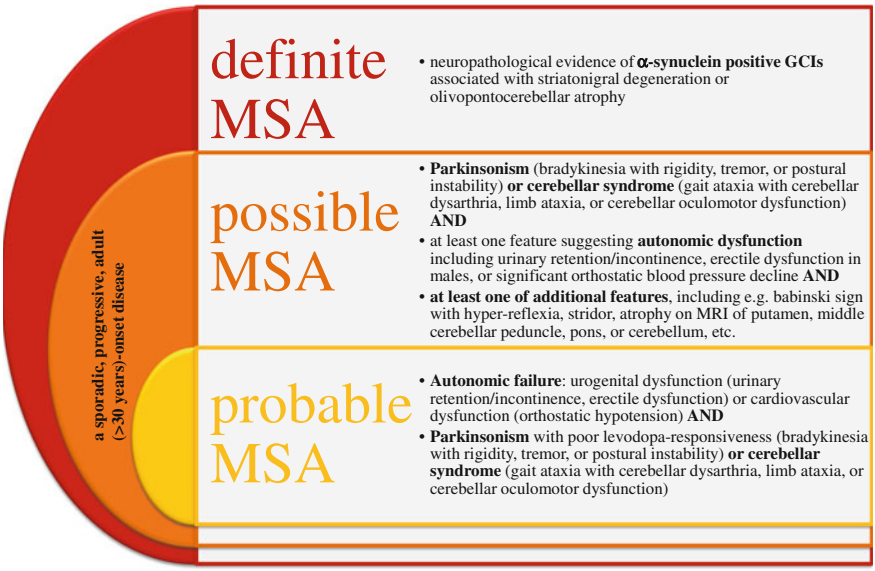

Fig. 1 Consensus statement and criteria for the clinical diagnosis of MSA adapted from Gilman et al. (2008). Three different categories of increasing certainty were established to ease the diagnosis of MSA for clinicians. These include: possible, probable, and definite MSA which can be diagnosed by means of specific clinical features and postmortem neuropathological examination

rigidity, and tremor, whereas cerebellar dysfunction is associated with gait ataxia, ataxic dysarthria, limb ataxia, and sustained gaze evoked nystagmus (Gilman et al. 1998). Ethnic variations regarding the incidence of MSA-P and MSA-C were found in epidemiological studies of Europe, North America, and Japan. A predominance of MSA-P was found for Europe and North America, where about 60 % of patients develop MSA-P (Gilman et al. 2005; Geser et al. 2006; May et al. 2007). However, in the Japanese population MSA-C is the more common MSA subtype with an incidence of about 83.8 % (Yabe et al. 2006). The cause of this variability remains unclear, but the involvement of environmental and/or genetic factors is suggested (Ubhi et al. 2011). In both forms of MSA the development of autonomic dysfunction, such as urogenital, gastrointestinal, and cardiovascular dysfunction, is common (Pfeiffer 2007; Ubhi et al. 2011). Furthermore, changes in behavior, including depression and executive dysfunction, may occur in MSA patients indicating an impairment of the frontal lobe (Fetoni et al. 1999; Dujardin et al. 2003; Benrud-Larson et al. 2005; Schrag et al. 2010).

Clinical diagnosis of MSA is based on the consensus statement that specifies the clinical domains, features, and criteria to define three diagnostic categories of increasing certainty: possible, probable, and definite MSA (Gilman et al. 2008). Possible and probable MSA are diagnosed by means of specific clinical features (see Fig. 1), whereas a definite diagnosis of MSA depends on postmortem

neuropathological evidence of glial cytoplasmic inclusions (GCIs) associated with SND or cerebellar ataxia (Trojanowski and Revesz 2007; Gilman et al. 2008). Furthermore, the unified MSA rating scale (UMSARS) was developed to help clinicians follow and assess disease progression in patients (Wenning et al. 2004b). Unfortunately, to date no treatment to stop the rapid disease progression exists. Only symptomatic treatment may be provided, however, treatment is moderately or poorly effective (Wenning et al. 2004a). A dopamine replacement therapy, i.e. levodopa, may be used to treat parkinsonism, yet only 28–65 % of pathological confirmed MSA cases presented with a positive response to levodopa treatment (Flabeau et al. 2010). Furthermore, in only 13 % of patients the effect of levodopa persists for some years and it may lead to pathological hypersexuality, the worsening of orthostatic hypotension, or early orofacial dyskinesias (Wenning et al. 1994; Klos et al. 2005). Other symptomatic treatments may be applied, including dopamine agonists (Wenning et al. 1994, 1997), amantadine (Wenning et al. 1997, 2005), and selective serotonin reuptake inhibitors (Friess et al. 2006) for the treatment of motor dysfunction, yet their efficiency is poor. Moreover, different treatments exist to alleviate autonomic symptoms, including, e.g., orthostatic hypotension, constipation, urinary retention, and breathing disorders [for review see (Flabeau et al. 2010)].

In contrast to genetic studies of PD/DLB revealing SNCA gene duplications, triplications as well as pathogenic point mutations (Polymeropoulos et al. 1997; Singleton et al. 2003; Zarranz et al. 2004; Nishioka et al. 2006), genetic studies in MSA did not reveal a mutation in the entire coding region of the α-synuclein (AS) gene (SNCA) locus (Ozawa et al. 1999). However, other studies demonstrated that polymorphisms within the SNCA locus may correlate with the risk of developing MSA (Al-Chalabi et al. 2009; Scholz et al. 2009), strengthening the assumption that AS processing plays a major role in the MSA pathogenesis. Yet, another research group could not replicate these findings due to high variability in the control group and furthermore, in a genome-wide association study in 2012, polymorphisms within the SNCA gene locus could not be confirmed either (Yun et al. 2010; Sailer 2012). Some MSA pedigrees consistent with Mendelian disease have been described, but the identification of a single gene failed (Hara et al. 2007; Wullner et al. 2009). Polymorphisms in some genes involved in inflammatory processes (e.g., genes encoding interleukins one and eight) were proposed to be associated with enhanced risk to develop MSA (Nishimura et al. 2002; Combarros et al. 2003; Infante et al. 2005). Another attempt to identify a gene involved in MSA etiology was undertaken in a genome-wide association study resulting in no definite finding (Sailer 2012). In a very recent study mutations in the gene *COQ2*, encoding the parahydroxybenzoate-polyprenyl transferase which is essential for the biosynthesis of the coenzyme Q10, were identified in some familial and sporadic MSA cases. These mutations leading to functional impairment of *COQ2* and therefore to an impairment of the mitochondrial respiratory chain and increased vulnerability to oxidative stress are suggested to be associated with an augmented risk of developing MSA (The Multiple-System Atrophy Research Collaboration 2013). These results lead to the assumption that genetic predisposition contributes

to MSA etiology, but in most cases non-genetic factors may play a major role maybe in interaction with susceptibility genes such as mutations in the *COQ2* gene (Jellinger 2012; The Multiple-System Atrophy Research Collaboration 2013).

Neuropathological examination of MSA brains reveals widespread neuronal loss in the striatum, substantia nigra pars compacta (SNpc), cerebellum, pons, inferior olives, and intermediolateral columns of the spinal cord (Stefanova et al. 2009a). Additionally, MSA brains exhibit prominent microglial and astroglial activation which may also play a role in the neurodegenerative process (Gerhard et al. 2003; Ishizawa et al. 2004; Ozawa et al. 2004). Moreover, the presence of argyrophilic filamentous GCIs in oligodendroglial cells throughout the brain is a major hallmark of MSA and was first described by Papp and colleagues (Papp et al. 1989). GCIs were found in pons, medulla, putamen, substantia nigra, cerebellum, and preganglionic autonomic structures (Papp and Lantos 1994; Beyer and Ariza 2007; Jellinger and Lantos 2010). The predominantly neuronal presynaptic protein (AS) was identified as major component of GCIs in 1998 (Spillantini et al. 1998; Wakabayashi et al. 1998). Therefore, the presence of AS-positive inclusions links MSA to PD and DLB, classifying them into the category of α-synucleinopathies (ASP). However, PD and DLB are identified as neuronal ASP due to AS-inclusions (Lewy bodies and Lewy neurites) occurring predominantly in neurons, whereas MSA is conceptualized as a primary oligodendrogliopathy or oligodendroglial ASP based on the presence of AS-positive aggregations mainly in oligodendroglial cells (Wenning et al. 2008; Fellner and Stefanova 2012). In addition to the aggregations in oligodendroglial cells, MSA brains feature neuronal and astroglial cytoplasmic AS-positive inclusions although in a decreased density (Wenning and Jellinger 2005).

In 2005, Jellinger and colleagues proposed a grading scale of MSA neuropathology by taking into account semiquantitative analyses of brain atrophy, neuronal loss, astrogliosis, and GCI pathology in different brain regions (Jellinger et al. 2005). Correlation of GCIs density in MSA brains with the disease duration and the degree of neuronal loss was established, thereby supporting the putative crucial role of oligodendroglial pathology in MSA (Ozawa et al. 2004). Interestingly, it was also found that the AS load is increased in MSA brains compared to PD and DLB (Tong et al. 2010).

GCIs present with different shapes, such as oval, sickle-shaped, or conical (Wenning and Jellinger 2005). Furthermore, GCIs were found to be immunoreactive to different other constituents in addition to the main component AS. These include tau, tubulin, ubiquitin, αB-crystallin, leucin-rich repeat serine/threonine-protein LRRK2, heat shock proteins, and prion disease-linked 14-3-3 protein among others (Wenning et al. 2008). The formation of AS-positive inclusions in oligodendroglial cells in MSA has not been fully elucidated yet. Two hypotheses on the formation of AS-positive inclusions exist: (1) the active uptake of AS by oligodendroglia or (2) selective upregulation of AS expression and slow degradation of AS in oligodendroglial cells (Fellner et al. 2011). Different aspects of recent research favor the first hypothesis, including that no AS mRNA expression has been detected in oligodendroglial cells of human control and MSA brains

(Ozawa et al. 2001; Miller et al. 2005). Moreover, recent data provide evidence that cell-to-cell propagation of AS may be the mechanism of AS aggregation in ASP (Desplats et al. 2009; Lee et al. 2010; Luk et al. 2012). In different experiments, release of AS into the extracellular space (Emmanouilidou et al. 2010) and furthermore the uptake of AS into neurons and astroglial cells in vivo and in vitro were demonstrated (Desplats et al. 2009; Luk et al. 2009, 2012; Lee et al. 2010; Hansen et al. 2011; Fellner et al. 2013). However, transmission of AS to oligodendroglial cells has not been proven in any in vivo graft experiment to date (Stefanova et al. 2009b; Hansen et al. 2011). In recent experiments the uptake of AS by oligodendroglial cell lines and primary rat oligodendroglia in a time- and concentration-dependent manner was suggested (Kisos et al. 2012; Konno et al. 2012). Yet, further research is needed to elucidate the formation of AS-positive inclusion in oligodendroglial cells in the brains of MSA patients. Importantly, a recent study by the group of Stanley Prusiner (Watts et al. 2013) experimentally demonstrated that AS aggregates present in MSA brains are transmissible and may induce lethal disease in transgenic mice.

Although different pathomechanisms in MSA have been suggested, no effective treatment to stop neurodegeneration has been found to date. Symptomatic treatment may be provided to alleviate symptoms, but it remains moderately or poorly effective (Wenning et al. 2004a; Low and Singer 2008; Kollensperger et al. 2010). The lack of treatment as well as the limited understanding of the pathobiological mechanisms called for a development of animal models of MSA. Many of these models were used as pre-clinical test beds for new therapeutic approaches or to explore different pathogenic processes.

The use of neurotoxins was one of the first approaches to mimic the disease pathology especially in rodents, but also in nonhuman primates. The use of stereotaxic or systemic injections of neurotoxins leads to a combined deterioration of the nigrostriatal system mimicking L-DOPA unresponsive Parkinsonism. In addition to the neurotoxic models, transgenic models were developed to create the core pathology of MSA, oligodendroglial AS-positive inclusions respectively. These genetic models are interesting tools to investigate the underlying mechanisms of neurodegeneration caused by abnormal oligodendroglial aggregation of AS and secondary neuronal dysfunction.

2 First Steps: The Replication of SND

Neurotoxin models were established to generate SND, the key neuropathology of MSA-P. Based on the knowledge of PD and Huntington's disease modeling through selective toxins, nigra and striatal toxins in combinations were used to create double-lesion SND/MSA-P models (Stefanova et al. 2005b). These double-lesion models reproduced L-DOPA unresponsive Parkinsonism typical for MSA-P. Two types of neurotoxin approaches can be distinguished: stereotaxic and systemic models.

The stereotaxic method induces a simultaneous or sequential unilateral degeneration of the SNpc and striatum and produces a dopamine unresponsive motor phenotype (Stefanova et al. 2005b; Fernagut and Tison 2012). The most widely used unilateral model applies 6-hydroxydopamine (6-OHDA) injected into the medial forebrain bundle (MFB) followed by quinolinic acid (QA) administrated into the striatum ipsilaterally (Wenning et al. 1996). This double lesion model presents with nigra and striatal neuronal loss, astrogliosis, microglial activation, and impaired motor behavior including amphetamine-induced ipsilateral rotations (but no apomorphine-induced rotation), severe impairment in paw-reaching and stepping tasks, side falling, and reduced overall activity in open field tests (Wenning et al. 1996; Scherfler et al. 2000, 2005; Stefanova et al. 2004b; Mantoan et al. 2005). The motor impairment does not respond to pulsatile L-DOPA administration, which can however induce dyskinetic behaviors (Stefanova et al. 2004a). It was found that the effect of the neurotoxins depended on the sequence of neurotoxin injections. The injection of 6-OHDA into the MFB prior to striatal lesion with QA weakened the neurotoxic effects of QA to the striatum as compared to animals with primary QA lesions (Scherfler et al. 2000). This well-characterized unilateral sequential double-toxin double-lesion rat model has been especially useful to test the role of neurotransplantation in SND.

A unilateral simultaneous injection of QA and 6-OHDA into the striatum was proposed to overcome the decreased vulnerability of striatal neurons due to preceding dopamine depletion (Ghorayeb et al. 2001). This strategy resulted in significant striatal degeneration due to QA, whereas the 6-OHDA-induced neuronal loss in the nigra region was less marked compared to 6-OHDA injection into the striatum alone suggesting that QA partly prevented retrograde dopaminergic denervation. In this simultaneous model astrogliosis was found, and behavioral analyses revealed reduced ipsilateral amphetamine-induced rotational behavior, attenuated contralateral apomorphine-induced rotational behavior compared to primary 6-OHDA or QA lesions, and severe impairment of paw reaching. The simultaneous strategy was suggested to serve as a model of mild, early SND that might qualify for early therapeutic strategies (Ghorayeb et al. 2001). A recent approach to mimic early MSA-P pathology applied a sequential striatal double-lesion approach in rats, whereby a partial 6-OHDA striatal lesion was followed by a QA injection into the striatum. The model presented severe nigral and moderate striatal degeneration combined with robust motor deficits (Kaindlstorfer et al. 2012).

Another strategy to model SND was the development of a unilateral "single toxin–double lesion" SND model to overcome the protection of striatal neurons as a result of a prior nigral lesion. For the generation of the lesions either the succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NP) (Waldner et al. 2001) or the mitochondrial complex I inhibitor 1-methyl-4-phenylpyridinium ion (MPP^+) (Ghorayeb et al. 2002a) were used. Both toxins when applied stereotaxically in the striatum cause neuronal loss in SNpc, extensive degeneration in the striatum, and astrogliosis. Moreover, motor impairment was also described for these stereotaxic models, including drug-induced rotation and severe deficits in paw reaching (Waldner et al. 2001; Ghorayeb et al. 2002a). In addition, MPP^+ treated animals

developed major motor deficits in side falling and thigmotactic scanning (Ghorayeb et al. 2002a).

The development of different degrees of nigral and/or striatal neuronal loss to model various stages of SND represents an important advantage of the stereotaxic rat models. Furthermore, they are an interesting tool to investigate various therapeutic strategies, including the evaluation of embryonic grafts as a possible therapy for MSA-P patients with the goal to achieve regeneration of L-DOPA responsiveness (Stefanova et al. 2005b). However, the stereotaxic rat models do not reproduce the pathological hallmark of MSA, namely AS-positive oligodendroglial inclusions, which is a major limitation of these models.

In addition to the unilateral stereotaxic models, systemic models were developed to mimic bilateral SND common for the human disease. The sub-chronic or chronic intoxication with systemic toxins induces a progressive neuronal dysfunction and temporal neurodegeneration similar to the development of neuronal deficits in MSA patients. SND in nonhuman primates (*Macaca fascicularis*) was modeled using intraperitoneal injections of the neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP) and 3-NP (Ghorayeb et al. 2000, 2002b). Thereby, MPTP treatment of monkeys was followed by administration of 3-NP inducing Parkinsonism with poor L-DOPA response, hind limb dystonia, as well as SND (Ghorayeb et al. 2000, 2002b). Systemic application of 3-NP in mice led to striatal neurodegeneration and mild loss of dopaminergic neurons associated with an impaired motor phenotype such as hind limb dystonia and clasping (Fernagut et al. 2002). Furthermore, the sequential administration of MPTP and 3-NP (MPTP prior to 3-NP and vice versa) in mice resulted in significant SND, astrogliosis and locomotor deficits. MPTP followed by 3-NP treatment decreased striatal damage, while prior 3-NP administration reduced MPTP-induced nigral degeneration (Stefanova et al. 2003). In a next step, a bilateral, simultaneous double-toxin double-lesion mouse model was developed to decrease the possibility of reduced neuronal vulnerability due to the sequential administration of MPTP and 3-NP. Thereby, the combined administration of MPTP and 3-NP induced striatal and nigral neuronal loss, as well as astrogliosis. The treated animals also showed disturbed balance, altered gait pattern, hindlimb and truncal dystonia, and severe motor symptoms including impairment in rotarod test, pole test, and traversing a beam (Fernagut et al. 2004; Diguet et al. 2005). Similar to the stereotaxic models, systemic neurotoxin models display different degrees of SND. Yet, they fail to reproduce the oligodendroglial inclusion pathology of MSA brains.

3 Reproducing the Specific Oligodendroglial AS Pathology of MSA in Transgenic Mice

As mentioned above, a disadvantage of the neurotoxin models is the lack of the core AS pathology of MSA (GCIs) which may play a fundamental role in the pathogenic mechanisms leading to neurodegeneration. Furthermore, these toxin

models cannot reproduce various other cardinal features of MSA, including autonomic and cerebellar dysfunction. The finding that AS and its aggregation in oligodendroglia are strongly involved in the pathology and the categorization of MSA into the group of ASP increases the necessity to investigate the AS-dependent pathophysiological mechanisms of the disorder. Therefore, the generation of a transgenic mouse model characterized by the overexpression of human wild-type AS under a specific oligodendroglial promoter has been a major step forward to the elucidation of the pathogenic mechanisms linked to the AS aggregation in oligodendroglial cells.

The first transgenic MSA mouse model was introduced in 2002 by Kahle and colleagues (Kahle et al. 2002). Targeted AS overexpression in oligodendroglial cells was achieved using the proteolipid-protein (PLP) promoter. Hyperphosphorylation at serine 129 and insolubility of AS were found in this transgenic mouse, similar to the human disease (Kahle et al. 2002). Moderate dopaminergic neuronal loss in SNpc of the PLP-AS mice was associated with a reduced stride length in aged transgenic mice (Stefanova et al. 2005a). Furthermore, the PLP-AS overexpressing mouse model presented with microglial activation (Stefanova et al. 2007) similar to the human disease (Gerhard et al. 2003). In addition to the progressive motor phenotype the PLP-AS mouse model showed also features of autonomic dysfunction: (1) cardiovascular autonomic dysfunction was linked to degeneration in brainstem nuclei involved in autonomic control (Stemberger et al. 2010; Kuzdas et al. 2013), and (2) bladder dysfunction was linked to neuronal loss in the pontine micturition center as well as loss of parasympathetic preganglionic neurons in the intermediolateral columns and loss of motor neurons in the Onuf's nucleus of the spinal cord (Boudes et al. 2013).

Another approach to generate oligodendroglial AS-overexpressing transgenic mice involved the application of the 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) promoter (Yazawa et al. 2005). The expression of AS in oligodendroglial cells led to axonal degeneration, brain atrophy, accumulation of endogenous mouse AS in axons and axon terminals predominantly in the spinal cord, astrogliosis, as well as neuronal and oligodendroglial loss in the spinal cord. Motor impairment was measured by a progressive reduction of rotarod performance (Yazawa et al. 2005). Moreover, in this model endogenous AS accumulation in neurons was dependent on the microtubule β-III tubulin protein and interfering with the synaptic vesicle release in GABAergic interneurons (Nakayama et al. 2009, 2012; Ito et al. 2012). Yet, the CNP-AS transgenic model could not replicate the selective neuropathology of MSA.

Overexpression of AS under the specific oligodendroglial promoter myelin basic protein (MBP) in mice was introduced by Shults and colleagues (Shults et al. 2005). The transgenic mice developed widespread insoluble human AS positive inclusion pathology characterized by phosphorylation of AS at serine 129. Furthermore, the MBP transgenic mice featured significant loss of dopaminergic terminals in striatum associated with an impaired motor phenotype as measured by rotarod and pole test (Shults et al. 2005). Reduced levels of several neurotrophic factors were found in the MBP-AS mouse model but not in neuronal overexpressors of AS, suggesting

that AS expression in oligodendroglia might impact neuronal trophic support inducing MSA-like neurodegeneration (Ubhi et al. 2010).

MSA transgenic models recapitulate the hallmark pathology of the human disease, namely AS-positive inclusions in oligodendroglial cells and also other features of MSA, such as microglial activation and autonomic failure. Therefore, these mouse models are highly useful for studies on pathogenic mechanisms of neurodegeneration in MSA, as well as for testing candidate therapeutic interventions.

4 Getting Insights into the Pathogenic Pathways of MSA Through the Application of Animal Models

Mitochondrial dysfunction and MSA

As mentioned before, most likely both genetic and environmental factors contribute to the etiopathogenesis of MSA. The first attempt to combine genetic predisposition and environmental risk factors in a mouse model was achieved in 2005 providing an animal model presenting a full-blown MSA pathology (Stefanova et al. 2005a). The transgenic mouse overexpressing AS in oligodendroglia was exposed to chronic oxidative stress [mitochondrial inhibition relevant to the recently found COQ2 mutation in MSA cases (The Multiple-System Atrophy Research Collaboration 2013)] induced by intraperitoneal injections of 3-NP. These transgenic mice treated with 3-NP presented with widespread AS inclusion pathology in oligodendroglia associated with SND and olivopontocerebellar atrophy similar to the human disorder. In wild-type control mice treated with 3-NP only mild SND was found, but no OPCA, suggesting a major involvement of AS pathology in MSA-like neurodegeneration. Furthermore, mitochondrial inhibition induced profound astrogliosis and microgliosis in the brains of transgenic mice. The animals also presented with augmented motor and behavioral deficits as demonstrated by hindlimb and trunk dystonia, as well as decreased horizontal and vertical locomotor activity, impaired pole test performance, and shortened stride length (Stefanova et al. 2005a, b). A similar approach was conducted in the mouse overexpressing human AS under the MBP promoter. Systemic 3-NP application augmented neurodegeneration and motor deficits and moreover, altered levels of oxidized and nitrated AS were found in this combined model (Ubhi et al. 2009).

Proteolytic failure and MSA

Recently, an involvement of the ubiquitin–proteasome system in the pathology of MSA was tested experimentally in the MSA transgenic mouse overexpressing AS under the PLP promoter (Stefanova et al. 2012b). The ubiquitin–proteasome system is responsible for the degradation of unneeded or damaged proteins. Alterations in the ubiquitin–proteasome system may contribute to the formation of AS aggregations in ASP (Tofaris et al. 2003; Ebrahimi-Fakhari et al. 2011). Proteolytic failure induced by systemic proteasome inhibition (PSI) in transgenic MSA mice triggered impaired open field motor behavior associated to progressive

SND and olivopontocerebellar neuronal degeneration. In contrast, PSI administration did not induce neurodegeneration or behavioral alterations in wild-type mice. Moreover, an increase of fibrillar human AS in the cytoplasm of oligodendroglia was found to lead to myelin disruption and demyelination in the PSI transgenic mice. Oligodendroglial dysfunction was followed by axonal degeneration (Stefanova et al. 2012b). These new data support the hypothesis that impaired protein degradation may play a major role in MSA pathology suggesting that a failure of the proteolytic system in the presence of oligodendroglial AS may induce MSA-like neurodegeneration.

Microglial activation and MSA

Increasing evidence suggests a role of microglial activation linked to AS pathology in the pathogenesis of MSA (Gerhard et al. 2003; Ishizawa et al. 2004; Fellner et al. 2013). Moreover, early progressive microglial activation was found to be associated with dopaminergic neuronal loss (Stefanova et al. 2007). Toll-like receptors (TLRs) are primarily expressed on cells of the innate immune system including microglia and they are important for the identification of conserved structural motifs on a wide array of pathogens (pathogen-associated molecular patterns) and for the recognition of endogenous molecules, including AS (Akira 2001). An upregulation of TLR4 was found in MSA brains and also MSA transgenic mice suggesting an involvement of TLR4 in the MSA pathogenesis (Stefanova et al. 2007). To identify the role of TLR4 in MSA-like ASP the PLP-AS overexpressing mouse model was crossbred with TLR4-deficient mice (Stefanova et al. 2011). TLR4 deficiency in MSA transgenic mice led to increased AS levels in the brains associated with augmented motor disability and enhanced loss of nigrostriatal dopaminergic neurons. Moreover, it was shown that enhanced AS levels were linked to disturbed microglial phagocytosis of AS mediated by TLR4 and to augmented tumor necrosis factor α (TNFα) release by astroglial cells (Stefanova et al. 2011; Fellner et al. 2013). These data reveal the importance of TLR4 in the clearance of AS by microglial cells and suggest that the upregulation of TLR4 may act as innate neuroprotective mechanism in MSA and other ASP (Letiembre et al. 2009).

In summary, transgenic MSA models emphasize the relevance of abnormal AS accumulation in oligodendroglial cells as a major key player in the pathogenesis of MSA. Moreover, different pathogenetic pathways, including mitochondrial dysfunction, impaired protein degradation, and microglial activation, are identified to be involved in disease mechanisms.

5 Screening Novel Therapeutic Interventions in MSA Animal Models

In light of the limited understanding of the pathomechanisms of MSA and the lack of effective treatments to slow down the rapid progression of the disease, the different animal models provide an invaluable tool to address these issues in

pre-clinical conditions. Thereby, cell- and drug-based therapeutic approaches can be distinguished. Some of those therapeutic interventions were found to improve behavior and reduce neuronal loss in the described MSA animal models. Therefore, the successful treatments were transferred into clinical trials to test their efficacy in MSA patients.

5.1 Cell-Based Therapeutic Approaches

Neurorestorative approaches by transplantation of fetal allografts into the striatum aim to counterfeit the loss of dopaminergic neurons and to restore the responsiveness to L-DOPA. Especially, double-lesion animal models have been beneficial to evaluate the functionality of embryonic grafts as a probable therapeutic strategy for MSA-P patients (Stefanova et al. 2005b). These studies suggest striatal transplantation as possible therapeutic option for MSA-P patients to restore the lacking L-DOPA response and thus improve the symptomatic treatment of the motor symptoms (Wenning et al. 1996; Puschban et al. 2005; Kollensperger et al. 2009). However, the transplantation of a striatal allograft into the PLP-AS overexpressing mouse model of MSA intoxicated with 3-NP presented with reduced dopaminergic re-innervation and p-zone volume suggesting that the presence of MSA-like AS oligodendrogliopathy compromises the neurorestorative outcome of the graft (Stefanova et al. 2009b). The variable outcomes of different studies using embryonic grafts for striatal transplantation in MSA may be due to the different experimental settings and rodent models used. The neurorestorative potential of embryonic neuronal allografts remains therefore unclear. A standardized protocol and an increased number of experimental studies may help to evaluate the beneficial effects of neurorestorative approaches in MSA.

Furthermore, intravenous infusion of mesenchymal stem cells (MSCs) was tested as a therapeutic strategy in MSA rodent models. In a mouse model of double-toxin (MPTP and 3-NP) - induced MSA, human MSCs induced neuronal protection in SN and striatum associated with behavioral improvements (Park et al. 2011). Park and colleagues proposed that a great number of human MSCs invade the CNS and may exert neuroprotection by modulation of inflammation, cell survival and cell death signaling-pathways (Park et al. 2011). In a different study, intravenous infusion of mouse MSCs in a MSA transgenic mouse model resulted in neuroprotection in SNpc and immunomodulatory effects in the brain although no MSC invasion was found. Furthermore, no behavioral improvement was detected (Stemberger et al. 2011). In patients with MSA-C the intraarterial and intravenous injection of autologous MSCs slowed transiently the disease progression (Lee et al. 2008, 2012), thus suggesting MSCs as a potential therapy for MSA. Due to the fact that the study conducted in 2008 was an open-label trial and therefore earned critics on the strength of the clinical evidence, Lee and colleagues performed a randomized double-blind MSC study in 2012 to confirm the positive outcome from 2008 (Lee et al. 2008, 2012) (Table 1). However, further studies

Table 1 List of drugs and treatments tested in pre-clinical and clinical trials

Drug/ Treatment	Pre-clinical studies	Clinical trial
Minocycline	Toxin MSA model (Stefanova et al. 2004b) anti-inflammatory, no neuroprotection Transgenic MSA model (Stefanova et al. 2007) anti-inflammatory, neuroprotection by early application	MEMSA-trial (Dodel et al. 2010) anti-inflammatory, no change in progression
Riluzole	Toxin model (Diguet et al. 2005; Scherfler et al. 2005) partial striatal neuroprotection, no motor improvement	NNIPPS study (Bensimon et al. 2009) no neuroprotection
Rifampicin	Transgenic MSA model (Ubhi et al. 2008) protective	RDCRC (clinicaltrials.gov NCT01287221) no neuroprotection
Rasagiline	Transgenic MSA model (Stefanova et al. 2008) protective	Multi-center study (Poewe et al. 2012) no change in progression
Fluoxetine	Transgenic MSA model (Ubhi et al. 2012) protective	French clinical trial (clinicaltrial.gov NCT01146548) negative outcome
MSCs	Toxin MSA model (Park et al. 2011) Transgenic MSA model (Stemberger et al. 2011) protective, immunomodulatory	South Korean clinical trial (Lee et al. 2008, 2012) delayed progression

Summary of putative neuroprotective and neuroimmunomodulatory treatments that were tested in pre-clinical studies. Due to the positive outcome in pre-clinical studies, clinical trials were conducted with the listed promising target drugs, yet the positive results gained in animal studies could not be replicated in MSA patients. Only the Korean study using mesenchymal stem cells (MSCs) could replicate the positive outcome of the pre-clinical trials. MEMSA-trial, Minocycline European Multiple System Atrophy-trial; NNIPPS, Neuroprotection and Natural History in Parkinson Plus Syndromes; RDCRC, Rare Diseases Clinical Research Consortia

have to be performed to identify the mechanisms underlying these effects and to replicate the results of Lee and colleagues in a different population cohort or in MSA-P patients.

5.2 Neuroprotective Strategies

The accumulation of AS has been identified as a critical step in the pathogenesis of MSA (Wenning et al. 2008; Stefanova et al. 2009a; Jellinger and Lantos 2010). Yet, the exact mechanisms leading to progressive neurodegeneration in MSA still need to be elucidated. Different aspects, linked to the neuronal loss in MSA have to be taken into account, including the toxicity of AS, AS accumulation in oligodendroglial cells as well as oligodendroglial dysfunction, oxidative stress and neuroinflammatory processes (e.g., microgliosis, astrogliosis). The development of transgenic animals overexpressing AS in oligodendroglial cells allows the screening for candidate drugs before introducing them in clinical trials (Flabeau et al. 2010). Currently several drugs have completed both pre-clinical and clinical

testing as listed below (and in Table 1). In spite of the negative clinical outcomes till date, these translational studies have been of importance to identify translational pitfalls and improve the design of the pre-clinical experiments with relevance to the clinics.

Minocycline is a tetracycline antibiotic with anti-inflammatory and anti-apoptotic properties that successfully crosses the blood–brain barrier (BBB) (Wang et al. 2003). Minocycline significantly decreased glial activation, but no neuroprotective effects were observed in a double-lesion rat model of SND (Stefanova et al. 2004b). However, early long-term treatment of PLP-AS transgenic mice with minocycline revealed protection of dopaminergic nigral neurons linked to decreased microglial activation (Stefanova et al. 2007). Parallel to the animal studies a randomized, double blind clinical trial with minocycline in MSA patients was performed. Similar to the animal experiments a significant reduction of microglial activation upon minocycline treatment was found as shown by [^{11}C](R)-PK11195 PET, but no clinical effect on symptom severity was observed, probably due to the late start of the treatment in already advanced MSA cases (Dodel et al. 2010). Minocycline seems to be a promising agent to stop microglial inflammatory processes early in the disease progression leading to a potential rescue of dopaminergic neurons. However, late diagnosis of MSA is a pitfall for this kind of treatment. Another anti-inflammatory approach is the inhibition of the myeoloperoxidase (MPO), an enzyme involved in production of ROS by phagocytic cells, including microglia (Reynolds et al. 1999). An involvement of MPO in the pathogenesis of neurodegenerative diseases, such as PD and HD, was suggested recently (Choi et al. 2005). In the combined PLP-AS + 3-NP MSA mouse model the early inhibition of MPO, using the MPO-inhibitor 1-(2-Isopropoxyethyl)-2-thioxo-1,2,3,5-tetrahydropyrrolo[3,2-d]pyrimidin-4-one, ameliorated motor deficits which were associated to neuroprotection in striatum and SNpc, cerebellar cortex, pontine nuclei, and inferior olives (Stefanova et al. 2012a). It remains to be identified whether late-start therapy with MPOi may have the same protective potency.

The inhibition of AS aggregation is considered a candidate therapeutic approach relevant for the treatment of MSA. Rifampicin is an antibiotic routinely used for the treatment of tuberculosis and leprosy, which has shown propensity to lower AS fibrillization in vitro (Li et al. 2004). Rifampicin was tested on its ability to reduce AS aggregation and to act neuroprotective in a MBP-AS transgenic MSA mouse model (Li et al. 2004; Ubhi et al. 2008). Treatment with rifampicin lowered the aggregation of AS in the mouse brains resulting in reduced neuronal loss and suppressed astroglial activation (Ubhi et al. 2008). The promising effects of rifampicin were assessed in a clinical trial that started in 2011. The study has been completed; however the drug failed to demonstrate improvement or the tendency of improvement in MSA (clinicaltrials.gov NCT01287221; Philip Low, http://www.msaawareness.org). The ability to reduce AS aggregations by the antibiotic rifampicin is an interesting approach as especially AS fibrils are thought to be very toxic and increase microglial and astroglial activation (Lee et al. 2010; Fellner et al. 2013). The positive result of rifampicin in pre-clinical testing may be due to the high drug dose used which was too high for clinical use. For the translation of

animal studies to clinical trials the drug dose given to the animals should relate to a clinical relevant dose.

The development of vaccines against AS aggregation is another very prospective approach to reduce AS levels and to slow down neurodegeneration in ASP. Active and passive immunization approaches targeting AS revealed attenuated AS accumulation and neurodegeneration in a mouse model of PD with LB pathology (Masliah et al. 2005, 2011). Furthermore, these results led to the first clinical immunization against AS in a PD cohort which started in 2012 (Schneeberger et al. 2010, 2012). Immunization studies targeting AS may also be an interesting target to slow down the progression of MSA.

Another therapeutic intervention tested both in pre-clinical and clinical MSA studies is the use of rasagiline which is a selective irreversible monoamine oxidase-B (MAO-B) inhibitor with certain anti-apoptotic and neurotrophic activity (Youdim et al. 2003; Bar-Am et al. 2004; Blandini et al. 2004; Eliash et al. 2005). In the PLP-AS transgenic mouse model intoxicated with 3-NP, rasagiline improved motor deficits as shown by pole test and stride length test associated with significant neuroprotection in various brain regions, such as striatum, SNpc, cerebellar cortex, pontine nuclei, and inferior olives (Stefanova et al. 2008). This drug entered a randomized, double-blind, placebo-controlled clinical trial in 2009. The study was completed in 2012, yet rasagiline had no effect on symptom severity as measured by using the UMSARS rating scale (Poewe et al. 2012). Similar to rifampicin, a higher dose of rasagiline was administered in the mouse study compared to the clinical trial. This might provide one explanation for the negative outcome of the clinical study. For further pre-clinical studies, the usage of a relevant drug dose should be considered.

The investigation of neuroprotective effects of riluzole was performed in a double-lesion rat model of SND. Riluzole is an anti-glutamatergic agent which is used for the treatment of amyotrophic lateral sclerosis (ALS) thereby prolonging survival (Bensimon et al. 1994; Lacomblez et al. 1996). Furthermore, riluzole was found to have a neuroprotective potential by the direct inhibition of protein kinase C (Koh et al. 1999; Noh et al. 2000; Obinu et al. 2002; Cheah et al. 2010; Carbone et al. 2012). In the double-lesion rat model of SND, riluzole treatment improved motor deficits and partially protected striatum (Scherfler et al. 2005). A similar result was achieved in a bilateral, simultaneous double-toxin double-lesion mouse model (using systemic intoxication with 3-NP and MPTP) treated with riluzole. Moderate behavioral improvements and neuroprotection were described in this mouse model (Diguet et al. 2005). Riluzole was also tested in a double-blind randomized placebo-controlled trial. However, no significant effect on survival or rate of functional deterioration in MSA was described (Bensimon et al. 2009). The achievement of a partial protection of the striatum is an interesting outcome in these animal studies; however, a partial pre-clinical protection proves not enough for the translation of a therapy into a clinical study.

Another interventional drug in the field of neurodegeneration is the use of fluoxetine. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and commonly used as antidepressant (Ubhi et al. 2012). Moreover, fluoxetine is known for

its influence on levels of neurotrophic factors such as glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) (Mercier et al. 2004; Chang et al. 2010; Allaman et al. 2011) and for its pro-proliferative activity (Chang et al. 2010; Wang et al. 2011). The drug was also reported to protect against neuronal toxin-induced damage in 6-OHDA and MPTP models of PD (Suzuki et al. 2010; Chung et al. 2011). The effects of the target drug fluoxetine on neuronal loss and behavior was investigated in the MBP-AS transgenic mouse model lately. The administration of fluoxetine by gavage revealed neuroprotective effects in the basal ganglia, neocortex and hippocampus of these mice. Moreover, reduced behavioral deficits were observed (Ubhi et al. 2012). In parallel a clinical study on fluoxetine in MSA was conducted (see clinicaltrials.gov NCT01146548). However, the results have been negative (unpublished).

Various different neuroprotective therapies have been developed in recent years. Testing of the different drug candidates in pre-clinical test beds revealed promising effects, especially regarding neuroprotection. The positive effects on neuroprotection in animal models of MSA also improved the behavioral deficits common in these rodent models. Yet, none of the therapeutic drug approaches tested in clinical trials brought the desired effect in MSA patients. One reason that some of the clinical trials failed might be due to the inadequate drug dose used in pre-clinical studies compared to the drug dose applicable and used in clinical trials. Another reason might be that especially the neuroprotective or neuroimmunomodulatory treatments should be given in an early phase of the disease, yet MSA is usually diagnosed rather late when the progression and therefore neurodegeneration of the disease are advanced. Thus, the diagnosis of MSA has to be improved with regard to sensitivity and specificity. Furthermore, no specific cerebrospinal fluid or blood biomarkers exist to assess the effect of different neuroprotective drugs in clinical trials. However, these translational pitfalls may be helpful to develop pre-clinical study designs that are more relevant for clinical studies. An extended search for an effective therapy to slow the progression of MSA will be a challenge for researchers in the next years.

6 Conclusion

In the last few years, considerable progress has been made to extend the knowledge regarding the pathogenesis of MSA. The different animal models mimicking MSA substantially expedited the understanding of the mechanisms on the pathology and progression of this neurodegenerative disorder. Furthermore, animal models of MSA allowed the testing of numerous different promising therapeutic compounds. Without the results of these studies the application of these drugs in clinical trials would not have been possible. However, the outcome of most disease-modifying therapeutic strategies did not meet the expectations of researchers,

clinicians, and patients. Therefore, increased effort investigating different therapeutic interventions has to be accomplished to increase the survival of MSA patients and the quality of life.

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References

Adams RD, Vanbogaert L, Vandereecken H (1964) Striato-nigral degeneration. J Neuropathol Exp Neurol 23:584–608

Akira S (2001) Toll-like receptors and innate immunity. Adv Immunol 78:1–56

Al-Chalabi A, Durr A, Wood NW, Parkinson MH, Camuzat A, Hulot JS, Morrison KE, Renton A, Sussmuth SD, Landwehrmeyer BG, Ludolph A, Agid Y, Brice A, Leigh PN, Bensimon G (2009) Genetic variants of the alpha-synuclein gene SNCA are associated with multiple system atrophy. PLoS ONE 4(9):e7114

Allaman I, Fiumelli H, Magistretti PJ, Martin JL (2011) Fluoxetine regulates the expression of neurotrophic/growth factors and glucose metabolism in astrocytes. Psychopharmacology 216(1):75–84

Bar-Am O, Yogev-Falach M, Amit T, Sagi Y, Youdim MB (2004) Regulation of protein kinase C by the anti-Parkinson drug, MAO-B inhibitor, rasagiline and its derivatives. in vivo J Neurochem 89(5):1119–1125

Benrud-Larson LM, Sandroni P, Schrag A, Low PA (2005) Depressive symptoms and life satisfaction in patients with multiple system atrophy. Mov Disord 20(8):951–957

Bensimon G, Lacomblez L, Meininger V (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole study group. N Engl J Med 330(9):585–591

Bensimon G, Ludolph A, Agid Y, Vidailhet M, Payan C, Leigh PN (2009) Riluzole treatment, survival and diagnostic criteria in Parkinson plus disorders: the NNIPPS study. Brain 132(Pt 1): 156–171

Beyer K, Ariza A (2007) Protein aggregation mechanisms in synucleinopathies: commonalities and differences. J Neuropathol Exp Neurol 66(11):965–974

Blandini F, Armentero MT, Fancellu R, Blaugrund E, Nappi G (2004) Neuroprotective effect of rasagiline in a rodent model of Parkinson's disease. Exp Neurol 187(2):455–459

Boudes M, Uvin P, Pinto S, Voets T, Fowler CJ, Wenning GK, De Ridder D, Stefanova N (2013) Bladder dysfunction in a transgenic mouse model of multiple system atrophy. Mov Disord 28(3):347–355

Carbone M, Duty S, Rattray M (2012) Riluzole neuroprotection in a Parkinson's disease model involves suppression of reactive astrocytosis but not GLT-1 regulation. BMC Neurosci 13:38

Chang EA, Beyhan Z, Yoo MS, Siripattarapravat K, Ko T, Lookingland KJ, Madhukar BV, Cibelli JB (2010) Increased cellular turnover in response to fluoxetine in neuronal precursors derived from human embryonic stem cells. Int J Dev Biol 54(4):707–715

Cheah BC, Vucic S, Krishnan AV, Kiernan MC (2010) Riluzole, neuroprotection and amyotrophic lateral sclerosis. Curr Med Chem 17(18):1942–1999

Choi DK, Pennathur S, Perier C, Tieu K, Teismann P, Wu DC, Jackson-Lewis V, Vila M, Vonsattel JP, Heinecke JW, Przedborski S (2005) Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. J Neurosci 25(28):6594–6600

Chung YC, Kim SR, Park JY, Chung ES, Park KW, Won SY, Bok E, Jin M, Park ES, Yoon SH, Ko HW, Kim YS, Jin BK (2011) Fluoxetine prevents MPTP-induced loss of dopaminergic neurons by inhibiting microglial activation. Neuropharmacology 60(6):963–974

Combarros O, Infante J, Llorca J, Berciano J (2003) Interleukin-1A (-889) genetic polymorphism increases the risk of multiple system atrophy. Mov Disord 18(11):1385–1386

Déjérine J, Thomas A (1900) L'atrophie olivoponto-cérébelleuse. Nouvelle Iconographie de la Salpêtiére 13:330–370

Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E, Lee SJ (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci USA 106(31):13010–13015

Diguet E, Fernagut PO, Scherfler C, Wenning G, Tison F (2005) Effects of riluzole on combined MPTP + 3-nitropropionic acid-induced mild to moderate striatonigral degeneration in mice. J Neural Transm 112(5):613–631

Dodel R, Spottke A, Gerhard A, Reuss A, Reinecker S, Schimke N, Trenkwalder C, Sixel-Doring F, Herting B, Kamm C, Gasser T, Sawires M, Geser F, Kollensperger M, Seppi K, Kloss M, Krause M, Daniels C, Deuschl G, Bottger S, Naumann M, Lipp A, Gruber D, Kupsch A, Du Y, Turkheimer F, Brooks DJ, Klockgether T, Poewe W, Wenning G, Schade-Brittinger C, Oertel WH, Eggert K (2010) Minocycline 1-year therapy in multiple-system-atrophy: effect on clinical symptoms and [(11)C] (R)-PK11195 PET (MEMSA-trial). Mov Disord 25(1):97–107

Dujardin K, Defebvre L, Krystkowiak P, Degreef JF, Destee A (2003) Executive function differences in multiple system atrophy and Parkinson's disease. Parkinsonism Relat Disord 9(4):205–211

Ebrahimi-Fakhari D, Cantuti-Castelvetri I, Fan Z, Rockenstein E, Masliah E, Hyman BT, McLean PJ, Unni VK (2011) Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of alpha-synuclein. J Neurosci 31(41):14508–14520

Eliash S, Shteter N, Eilam R (2005) Neuroprotective effect of rasagiline, a monoamine oxidase-B inhibitor, on spontaneous cell degeneration in a rat model. J Neural Transm 112(8):991–1003

Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, Stefanis L, Vekrellis K (2010) Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J Neurosci 30(20):6838–6851

Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, Wenning GK, Stefanova N (2013) Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. Glia 61(3):349–360

Fellner L, Jellinger KA, Wenning GK, Stefanova N (2011) Glial dysfunction in the pathogenesis of alpha-synucleinopathies: emerging concepts. Acta Neuropathol 121(6):675–693

Fellner L, Stefanova N (2013) The role of glia in alpha-synucleinopathies. Mol Neurobiol 47(2):575–586

Fernagut PO, Diguet E, Bioulac B, Tison F (2004) MPTP potentiates 3-nitropropionic acid-induced striatal damage in mice: reference to striatonigral degeneration. Exp Neurol 185(1):47–62

Fernagut PO, Diguet E, Stefanova N, Biran M, Wenning GK, Canioni P, Bioulac B, Tison F (2002) Subacute systemic 3-nitropropionic acid intoxication induces a distinct motor disorder in adult C57Bl/6 mice: behavioural and histopathological characterisation. Neuroscience 114(4):1005–1017

Fernagut PO, Tison F (2012) Animal models of multiple system atrophy. Neuroscience 211:77–82

Fetoni V, Soliveri P, Monza D, Testa D, Girotti F (1999) Affective symptoms in multiple system atrophy and Parkinson's disease: response to levodopa therapy. J Neurol Neurosurg Psychiatry 66(4):541–544

Flabeau O, Meissner WG, Tison F (2010) Multiple system atrophy: current and future approaches to management. Ther Adv Neurol Disord 3(4):249–263

Friess E, Kuempfel T, Modell S, Winkelmann J, Holsboer F, Ising M, Trenkwalder C (2006) Paroxetine treatment improves motor symptoms in patients with multiple system atrophy. Parkinsonism Relat Disord 12(7):432–437

Gerhard A, Banati RB, Goerres GB, Cagnin A, Myers R, Gunn RN, Turkheimer F, Good CD, Mathias CJ, Quinn N, Schwarz J, Brooks DJ (2003) [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. Neurology 61(5):686–689

Geser F, Wenning GK, Seppi K, Stampfer-Kountchev M, Scherfler C, Sawires M, Frick C, Ndayisaba JP, Ulmer H, Pellecchia MT, Barone P, Kim HT, Hooker J, Quinn NP, Cardozo A, Tolosa E, Abele M, Klockgether T, Ostergaard K, Dupont E, Schimke N, Eggert KM, Oertel W, Djaldetti R, Poewe W (2006) Progression of multiple system atrophy (MSA): a prospective natural history study by the European MSA Study Group (EMSA SG). Mov Disord 21(2):179–186

Ghorayeb I, Fernagut PO, Aubert I, Bezard E, Poewe W, Wenning GK, Tison F (2000) Toward a primate model of L-dopa-unresponsive parkinsonism mimicking striatonigral degeneration. Mov Disord 15(3):531–536

Ghorayeb I, Fernagut PO, Hervier L, Labattu B, Bioulac B, Tison F (2002a) A 'single toxin-double lesion' rat model of striatonigral degeneration by intrastriatal 1-methyl-4-phenylpyridinium ion injection: a motor behavioural analysis. Neuroscience 115(2):533–546

Ghorayeb I, Fernagut PO, Stefanova N, Wenning GK, Bioulac B, Tison F (2002b) Dystonia is predictive of subsequent altered dopaminergic responsiveness in a chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine + 3-nitropropionic acid model of striatonigral degeneration in monkeys. Neurosci Lett 335(1):34–38

Ghorayeb I, Puschban Z, Fernagut PO, Scherfler C, Rouland R, Wenning GK, Tison F (2001) Simultaneous intrastriatal 6-hydroxydopamine and quinolinic acid injection: a model of early-stage striatonigral degeneration. Exp Neurol 167(1):133–147

Gilman S, Low P, Quinn N, Albanese A, Ben-Shlomo Y, Fowler C, Kaufmann H, Klockgether T, Lang A, Lantos P, Litvan I, Mathias C, Oliver E, Robertson D, Schatz I, Wenning G (1998) Consensus statement on the diagnosis of multiple system atrophy. American autonomic society and American academy of neurology. Clin Auton Res 8(6):359–362

Gilman S, May SJ, Shults CW, Tanner CM, Kukull W, Lee VM, Masliah E, Low P, Sandroni P, Trojanowski JQ, Ozelius L, Foroud T (2005) The north American multiple system atrophy study group. J Neural Transm 112(12):1687–1694

Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Durr A, Fowler CJ, Kaufmann H, Klockgether T, Lees A, Poewe W, Quinn N, Revesz T, Robertson D, Sandroni P, Seppi K, Vidailhet M (2008) Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71(9):670–676

Graham JG, Oppenheimer DR (1969) Orthostatic hypotension and nicotine sensitivity in a case of multiple system atrophy. J Neurol Neurosurg Psychiatry 32(1):28–34

Hansen C, Angot E, Bergstrom AL, Steiner JA, Pieri L, Paul G, Outeiro TF, Melki R, Kallunki P, Fog K, Li JY, Brundin P (2011) alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J Clin Invest 121(2):715–725

Hara K, Momose Y, Tokiguchi S, Shimohata M, Terajima K, Onodera O, Kakita A, Yamada M, Takahashi H, Hirasawa M, Mizuno Y, Ogata K, Goto J, Kanazawa I, Nishizawa M, Tsuji S (2007) Multiplex families with multiple system atrophy. Arch Neurol 64(4):545–551

Infante J, Llorca J, Berciano J, Combarros O (2005) Interleukin-8, intercellular adhesion molecule-1 and tumour necrosis factor-alpha gene polymorphisms and the risk for multiple system atrophy. J Neurol Sci 228(1):11–13

Ishizawa K, Komori T, Sasaki S, Arai N, Mizutani T, Hirose T (2004) Microglial activation parallels system degeneration in multiple system atrophy. J Neuropathol Exp Neurol 63(1):43–52

Ito H, Nakayama K, Jin C, Suzuki Y, Yazawa I (2012) alpha-Synuclein accumulation reduces GABAergic inhibitory transmission in a model of multiple system atrophy. Biochem Biophys Res Commun 428(3):348–353

Jellinger KA (2012) The role of α-synuclein in neurodegeneration—an update. Translat Neurosci 3(2):75–122

Jellinger KA, Lantos PL (2010) Papp-Lantos inclusions and the pathogenesis of multiple system atrophy: an update. Acta Neuropathol 119(6):657–667

Jellinger KA, Seppi K, Wenning GK (2005) Grading of neuropathology in multiple system atrophy: proposal for a novel scale. Mov Disord 20(Suppl 12):S29–S36

Kahle PJ, Neumann M, Ozmen L, Muller V, Jacobsen H, Spooren W, Fuss B, Mallon B, Macklin WB, Fujiwara H, Hasegawa M, Iwatsubo T, Kretzschmar HA, Haass C (2002) Hyperphosphorylation and insolubility of alpha-synuclein in transgenic mouse oligodendrocytes. EMBO Rep 3(6):583–588

Kaindlstorfer C, Garcia J, Winkler C, Wenning GK, Nikkhah G, Dobrossy MD (2012) Behavioral and histological analysis of a partial double-lesion model of parkinson-variant multiple system atrophy. J Neurosci Res 90(6):1284–1295

Kisos H, Pukass K, Ben-Hur T, Richter-Landsberg C, Sharon R (2012) Increased neuronal alpha-synuclein pathology associates with its accumulation in oligodendrocytes in mice modeling alpha-synucleinopathies. PLoS ONE 7(10):e46817

Klos KJ, Bower JH, Josephs KA, Matsumoto JY, Ahlskog JE (2005) Pathological hypersexuality predominantly linked to adjuvant dopamine agonist therapy in Parkinson's disease and multiple system atrophy. Parkinsonism Relat Disord 11(6):381–386

Koh JY, Kim DK, Hwang JY, Kim YH, Seo JH (1999) Antioxidative and proapoptotic effects of riluzole on cultured cortical neurons. J Neurochem 72(2):716–723

Kollensperger M, Geser F, Ndayisaba JP, Boesch S, Seppi K, Ostergaard K, Dupont E, Cardozo A, Tolosa E, Abele M, Klockgether T, Yekhlef F, Tison F, Daniels C, Deuschl G, Coelho M, Sampaio C, Bozi M, Quinn N, Schrag A, Mathias CJ, Fowler C, Nilsson CF, Widner H, Schimke N, Oertel W, Del Sorbo F, Albanese A, Pellecchia MT, Barone P, Djaldetti R, Colosimo C, Meco G, Gonzalez-Mandly A, Berciano J, Gurevich T, Giladi N, Galitzky M, Rascol O, Kamm C, Gasser T, Siebert U, Poewe W, Wenning GK (2010) Presentation, diagnosis, and management of multiple system atrophy in Europe: final analysis of the European multiple system atrophy registry. Mov Disord 25(15):2604–2612

Kollensperger M, Stefanova N, Pallua A, Puschban Z, Dechant G, Hainzer M, Reindl M, Poewe W, Nikkhah G, Wenning GK (2009) Striatal transplantation in a rodent model of multiple system atrophy: effects on L-Dopa response. J Neurosci Res 87(7):1679–1685

Konno M, Hasegawa T, Baba T, Miura E, Sugeno N, Kikuchi A, Fiesel FC, Sasaki T, Aoki M, Itoyama Y, Takeda A (2012) Suppression of dynamin GTPase decreases alpha-synuclein uptake by neuronal and oligodendroglial cells: a potent therapeutic target for synucleinopathy. Mol Neurodegener 7:38

Kuzdas D, Stemberger S, Gaburro S, Stefanova N, Singewald N, Wenning GK (2013) Oligodendroglial alpha-synucleinopathy and MSA-like cardiovascular autonomic failure: experimental evidence. Exp Neurol 247:531–536

Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V (1996) Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis/riluzole study group II. Lancet 347(9013):1425–1431

Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, Hwang D, Masliah E, Lee SJ (2010) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285(12):9262–9272

Lee PH, Kim JW, Bang OY, Ahn YH, Joo IS, Huh K (2008) Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmacol Ther 83(5):723–730

Lee PH, Lee JE, Kim HS, Song SK, Lee HS, Nam HS, Cheong JW, Jeong Y, Park HJ, Kim DJ, Nam CM, Lee JD, Kim HO, Sohn YH (2012) A randomized trial of mesenchymal stem cells in multiple system atrophy. Ann Neurol 72(1):32–40

Letiembre M, Liu Y, Walter S, Hao W, Pfander T, Wrede A, Schulz-Schaeffer W, Fassbender K (2009) Screening of innate immune receptors in neurodegenerative diseases: a similar pattern. Neurobiol Aging 30(5):759–768

Li J, Zhu M, Rajamani S, Uversky VN, Fink AL (2004) Rifampicin inhibits alpha-synuclein fibrillation and disaggregates fibrils. Chem Biol 11(11):1513–1521

Low PA, Singer W (2008) Management of neurogenic orthostatic hypotension: an update. Lancet Neurol 7(5):451–458

Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, Lee VM (2012) Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338(6109):949–953

Luk KC, Song C, O'Brien P, Stieber A, Branch JR, Brunden KR, Trojanowski JQ, Lee VM (2009) Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci USA 106(47):20051–20056

Mantoan L, Stefanova N, Egger KE, Jellinger KA, Poewe W, Wenning GK (2005) Failure of caspase inhibition in the double-lesion rat model of striatonigral degeneration (multiple system atrophy). Acta Neuropathol 109(2):191–197

Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, Games D, Schenk D (2005) Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. Neuron 46(6):857–868

Masliah E, Rockenstein E, Mante M, Crews L, Spencer B, Adame A, Patrick C, Trejo M, Ubhi K, Rohn TT, Mueller-Steiner S, Seubert P, Barbour R, McConlogue L, Buttini M, Games D, Schenk D (2011) Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. PLoS ONE 6(4):e19338

May S, Gilman S, Sowell BB, Thomas RG, Stern MB, Colcher A, Tanner CM, Huang N, Novak P, Reich SG, Jankovic J, Ondo WG, Low PA, Sandroni P, Lipp A, Marshall FJ, Wooten F, Shults CW (2007) Potential outcome measures and trial design issues for multiple system atrophy. Mov Disord 22(16):2371–2377

Mercier G, Lennon AM, Renouf B, Dessouroux A, Ramauge M, Courtin F, Pierre M (2004) MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. J Mol Neurosci 24(2):207–216

Miller DW, Johnson JM, Solano SM, Hollingsworth ZR, Standaert DG, Young AB (2005) Absence of alpha-synuclein mRNA expression in normal and multiple system atrophy oligodendroglia. J Neural Transm 112(12):1613–1624

Nakayama K, Suzuki Y, Yazawa I (2009) Microtubule depolymerization suppresses alpha-synuclein accumulation in a mouse model of multiple system atrophy. Am J Pathol 174(4):1471–1480

Nakayama K, Suzuki Y, Yazawa I (2012) Binding of neuronal alpha-synuclein to beta-III tubulin and accumulation in a model of multiple system atrophy. Biochem Biophys Res Commun 417(4):1170–1175

Nishimura M, Kawakami H, Komure O, Maruyama H, Morino H, Izumi Y, Nakamura S, Kaji R, Kuno S (2002) Contribution of the interleukin-1beta gene polymorphism in multiple system atrophy. Mov Disord 17(4):808–811

Nishioka K, Hayashi S, Farrer MJ, Singleton AB, Yoshino H, Imai H, Kitami T, Sato K, Kuroda R, Tomiyama H, Mizoguchi K, Murata M, Toda T, Imoto I, Inazawa J, Mizuno Y, Hattori N (2006) Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. Ann Neurol 59(2):298–309

Noh KM, Hwang JY, Shin HC, Koh JY (2000) A novel neuroprotective mechanism of riluzole: direct inhibition of protein kinase C. Neurobiol Dis 7(4):375–383

O'Sullivan SS, Massey LA, Williams DR, Silveira-Moriyama L, Kempster PA, Holton JL, Revesz T, Lees AJ (2008) Clinical outcomes of progressive supranuclear palsy and multiple system atrophy. Brain 131(Pt 5):1362–1372

Obinu MC, Reibaud M, Blanchard V, Moussaoui S, Imperato A (2002) Neuroprotective effect of riluzole in a primate model of Parkinson's disease: behavioral and histological evidence. Mov Disord 17(1):13–19

Ozawa T, Okuizumi K, Ikeuchi T, Wakabayashi K, Takahashi H, Tsuji S (2001) Analysis of the expression level of alpha-synuclein mRNA using postmortem brain samples from pathologically confirmed cases of multiple system atrophy. Acta Neuropathol 102(2):188–190

Ozawa T, Paviour D, Quinn NP, Josephs KA, Sangha H, Kilford L, Healy DG, Wood NW, Lees AJ, Holton JL, Revesz T (2004) The spectrum of pathological involvement of the striatonigral

and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations. Brain 127(Pt 12):2657–2671

Ozawa T, Takano H, Onodera O, Kobayashi H, Ikeuchi T, Koide R, Okuizumi K, Shimohata T, Wakabayashi K, Takahashi H, Tsuji S (1999) No mutation in the entire coding region of the alpha-synuclein gene in pathologically confirmed cases of multiple system atrophy. Neurosci Lett 270(2):110–112

Papp MI, Kahn JE, Lantos PL (1989) Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). J Neurol Sci 94(1–3):79–100

Papp MI, Lantos PL (1994) The distribution of oligodendroglial inclusions in multiple system atrophy and its relevance to clinical symptomatology. Brain 117(Pt 2):235–243

Park HJ, Bang G, Lee BR, Kim HO, Lee PH (2011) Neuroprotective effect of human mesenchymal stem cells in an animal model of double toxin-induced multiple system atrophy parkinsonism. Cell Transplant 20(6):827–835

Pfeiffer RF (2007) Multiple system atrophy. Handb Clin Neurol 84:305–326

Poewe W, Barone P, Gliadi N, Gilman S, Low PA, Sampaio C, Seppi K (2012) A randomized, placebo-controlled clinical trial to assess the effects of rasagiline in patients with multiple system atrophy of the parkinsonian subtype [abstract]. Mov Disord 27(Suppl 1):1182

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276(5321):2045–2047

Puschban Z, Stefanova N, Petersen A, Winkler C, Brundin P, Poewe W, Wenning GK (2005) Evidence for dopaminergic re-innervation by embryonic allografts in an optimized rat model of the Parkinsonian variant of multiple system atrophy. Brain Res Bull 68(1–2):54–58

Reynolds WF, Rhees J, Maciejewski D, Paladino T, Sieburg H, Maki RA, Masliah E (1999) Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. Exp Neurol 155(1):31–41

Sailer A (2012) on behalf of the MSA GWAS Consortium. First genome-wide association study in multiple system atrophy [abstract]. Mov Disord 27 Suppl 1:1425

Scherfler C, Puschban Z, Ghorayeb I, Goebel GP, Tison F, Jellinger K, Poewe W, Wenning GK (2000) Complex motor disturbances in a sequential double lesion rat model of striatonigral degeneration (multiple system atrophy). Neuroscience 99(1):43–54

Scherfler C, Sather T, Diguet E, Stefanova N, Puschban Z, Tison F, Poewe W, Wenning GK (2005) Riluzole improves motor deficits and attenuates loss of striatal neurons in a sequential double lesion rat model of striatonigral degeneration (parkinson variant of multiple system atrophy). J Neural Transm 112(8):1025–1033

Schneeberger A, Mandler M, Mattner F, Schmidt W (2010) AFFITOME(R) technology in neurodegenerative diseases: the doubling advantage. Hum Vaccin 6(11):948–952

Schneeberger A, Mandler M, Mattner F, Schmidt W (2012) Vaccination for Parkinson's disease. Parkinsonism Relat Disord 18(Suppl 1):S11–S13

Scholz SW, Houlden H, Schulte C, Sharma M, Li A, Berg D, Melchers A, Paudel R, Gibbs JR, Simon-Sanchez J, Paisan-Ruiz C, Bras J, Ding J, Chen H, Traynor BJ, Arepalli S, Zonozi RR, Revesz T, Holton J, Wood N, Lees A, Oertel W, Wullner U, Goldwurm S, Pellecchia MT, Illig T, Riess O, Fernandez HH, Rodriguez RL, Okun MS, Poewe W, Wenning GK, Hardy JA, Singleton AB, Del Sorbo F, Schneider S, Bhatia KP, Gasser T (2009) SNCA variants are associated with increased risk for multiple system atrophy. Ann Neurol 65(5):610–614

Schrag A, Ben-Shlomo Y, Quinn NP (1999) Prevalence of progressive supranuclear palsy and multiple system atrophy: a cross-sectional study. Lancet 354(9192):1771–1775

Schrag A, Sheikh S, Quinn NP, Lees AJ, Selai C, Mathias C, Litvan I, Lang AE, Bower JH, Burn DJ, Low P, Jahanshahi M (2010) A comparison of depression, anxiety, and health status in patients with progressive supranuclear palsy and multiple system atrophy. Mov Disord 25(8):1077–1081

Schrag A, Wenning GK, Quinn N, Ben-Shlomo Y (2008) Survival in multiple system atrophy. Mov Disord 23(2):294–296

Shults CW, Rockenstein E, Crews L, Adame A, Mante M, Larrea G, Hashimoto M, Song D, Iwatsubo T, Tsuboi K, Masliah E (2005) Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. J Neurosci 25(46):10689–10699

Shy GM, Drager GA (1960) A neurological syndrome associated with orthostatic hypotension: a clinical-pathologic study. Arch Neurol 2:511–527

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) Alpha-synuclein locus triplication causes Parkinson's disease. Science 302(5646):841

Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M (1998) Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 251(3):205–208

Stefanova N, Bucke P, Duerr S, Wenning GK (2009a) Multiple system atrophy: an update. Lancet Neurol 8(12):1172–1178

Stefanova N, Fellner L, Reindl M, Masliah E, Poewe W, Wenning GK (2011) Toll-like receptor 4 promotes alpha-synuclein clearance and survival of nigral dopaminergic neurons. Am J Pathol 179(2):954–963

Stefanova N, Georgievska B, Eriksson H, Poewe W, Wenning GK (2012a) Myeloperoxidase inhibition ameliorates multiple system atrophy-like degeneration in a transgenic mouse model. Neurotox Res 21(4):393–404

Stefanova N, Hainzer M, Stemberger S, Couillard-Despres S, Aigner L, Poewe W, Wenning GK (2009b) Striatal transplantation for multiple system atrophy—are grafts affected by alpha-synucleinopathy? Exp Neurol 219(1):368–371

Stefanova N, Kaufmann WA, Humpel C, Poewe W, Wenning GK (2012b) Systemic proteasome inhibition triggers neurodegeneration in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. Acta Neuropathol 124(1):51–65

Stefanova N, Lundblad M, Tison F, Poewe W, Cenci MA, Wenning GK (2004a) Effects of pulsatile L-DOPA treatment in the double lesion rat model of striatonigral degeneration (multiple system atrophy). Neurobiol Dis 15(3):630–639

Stefanova N, Mitschnigg M, Ghorayeb I, Diguet E, Geser F, Tison F, Poewe W, Wenning GK (2004b) Failure of neuronal protection by inhibition of glial activation in a rat model of striatonigral degeneration. J Neurosci Res 78(1):87–91

Stefanova N, Poewe W, Wenning GK (2008) Rasagiline is neuroprotective in a transgenic model of multiple system atrophy. Exp Neurol 210(2):421–427

Stefanova N, Puschban Z, Fernagut PO, Brouillet E, Tison F, Reindl M, Jellinger KA, Poewe W, Wenning GK (2003) Neuropathological and behavioral changes induced by various treatment paradigms with MPTP and 3-nitropropionic acid in mice: towards a model of striatonigral degeneration (multiple system atrophy). Acta Neuropathol 106(2):157–166

Stefanova N, Reindl M, Neumann M, Haass C, Poewe W, Kahle PJ, Wenning GK (2005a) Oxidative stress in transgenic mice with oligodendroglial alpha-synuclein overexpression replicates the characteristic neuropathology of multiple system atrophy. Am J Pathol 166(3):869–876

Stefanova N, Reindl M, Neumann M, Kahle PJ, Poewe W, Wenning GK (2007) Microglial activation mediates neurodegeneration related to oligodendroglial alpha-synucleinopathy: implications for multiple system atrophy. Mov Disord 22(15):2196–2203

Stefanova N, Tison F, Reindl M, Poewe W, Wenning GK (2005b) Animal models of multiple system atrophy. Trends Neurosci 28(9):501–506

Stemberger S, Jamnig A, Stefanova N, Lepperdinger G, Reindl M, Wenning GK (2011) Mesenchymal stem cells in a transgenic mouse model of multiple system atrophy: immunomodulation and neuroprotection. PLoS ONE 6(5):e19808

Stemberger S, Poewe W, Wenning GK, Stefanova N (2010) Targeted overexpression of human alpha-synuclein in oligodendroglia induces lesions linked to MSA-like progressive autonomic failure. Exp Neurol 224(2):459–464

Suzuki K, Okada K, Wakuda T, Shinmura C, Kameno Y, Iwata K, Takahashi T, Suda S, Matsuzaki H, Iwata Y, Hashimoto K, Mori N (2010) Destruction of dopaminergic neurons in the midbrain by 6-hydroxydopamine decreases hippocampal cell proliferation in rats: reversal by fluoxetine. PLoS ONE 5(2):e9260

Tada M, Onodera O, Ozawa T, Piao YS, Kakita A, Takahashi H, Nishizawa M (2007) Early development of autonomic dysfunction may predict poor prognosis in patients with multiple system atrophy. Arch Neurol 64(2):256–260

The Multiple-System Atrophy Research Collaboration (2013) Mutations in COQ2 in familial and sporadic multiple-system atrophy. N Engl J Med 369(3):233–244

Tofaris GK, Razzaq A, Ghetti B, Lilley KS, Spillantini MG (2003) Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. J Biol Chem 278(45):44405–44411

Tong J, Wong H, Guttman M, Ang LC, Forno LS, Shimadzu M, Rajput AH, Muenter MD, Kish SJ, Hornykiewicz O, Furukawa Y (2010) Brain alpha-synuclein accumulation in multiple system atrophy, Parkinson's disease and progressive supranuclear palsy: a comparative investigation. Brain 133(Pt 1):172–188

Trojanowski JQ, Revesz T (2007) Proposed neuropathological criteria for the post mortem diagnosis of multiple system atrophy. Neuropathol Appl Neurobiol 33(6):615–620

Ubhi K, Inglis C, Mante M, Patrick C, Adame A, Spencer B, Rockenstein E, May V, Winkler J, Masliah E (2012) Fluoxetine ameliorates behavioral and neuropathological deficits in a transgenic model mouse of alpha-synucleinopathy. Exp Neurol 234(2):405–416

Ubhi K, Lee PH, Adame A, Inglis C, Mante M, Rockenstein E, Stefanova N, Wenning GK, Masliah E (2009) Mitochondrial inhibitor 3-nitroproprionic acid enhances oxidative modification of alpha-synuclein in a transgenic mouse model of multiple system atrophy. J Neurosci Res 87(12):2728–2739

Ubhi K, Low P, Masliah E (2011) Multiple system atrophy: a clinical and neuropathological perspective. Trends Neurosci 34(11):581–590

Ubhi K, Rockenstein E, Mante M, Inglis C, Adame A, Patrick C, Whitney K, Masliah E (2010) Neurodegeneration in a transgenic mouse model of multiple system atrophy is associated with altered expression of oligodendroglial-derived neurotrophic factors. J Neurosci 30(18):6236–6246

Ubhi K, Rockenstein E, Mante M, Patrick C, Adame A, Thukral M, Shults C, Masliah E (2008) Rifampicin reduces alpha-synuclein in a transgenic mouse model of multiple system atrophy. NeuroReport 19(13):1271–1276

Vanacore N, Bonifati V, Fabbrini G, Colosimo C, De Michele G, Marconi R, Nicholl D, Locuratolo N, Talarico G, Romano S, Stocchi F, Bonuccelli U, De Mari M, Vieregge P, Meco G (2001) Epidemiology of multiple system atrophy. ESGAP consortium. European study group on atypical Parkinsonisms. Neurol Sci 22(1):97–99

Wakabayashi K, Hayashi S, Kakita A, Yamada M, Toyoshima Y, Yoshimoto M, Takahashi H (1998) Accumulation of alpha-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy. Acta Neuropathol 96(5):445–452

Waldner R, Puschban Z, Scherfler C, Seppi K, Jellinger K, Poewe W, Wenning GK (2001) No functional effects of embryonic neuronal grafts on motor deficits in a 3-nitropropionic acid rat model of advanced striatonigral degeneration (multiple system atrophy). Neuroscience 102(3):581–592

Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, Ferrante RJ, Kristal BS, Friedlander RM (2003) Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proc Natl Acad Sci USA 100(18):10483–10487

Wang Y, Neumann M, Hansen K, Hong SM, Kim S, Noble-Haeusslein LJ, Liu J (2011) Fluoxetine increases hippocampal neurogenesis and induces epigenetic factors but does not improve functional recovery after traumatic brain injury. J Neurotrauma 28(2):259–268

Watts JC, Giles K, Oehler A, Middleton L, Dexter DT, Gentleman SM, Dearmond SJ, Prusiner SB (2013) Transmission of multiple system atrophy prions to transgenic mice. Proc Natl Acad Sci USA 110(48):19555–19560

Wenning GK (2005) Placebo-controlled trial of amantadine in multiple-system atrophy. Clin Neuropharmacol 28(5):225–227

Wenning GK, Ben Shlomo Y, Magalhaes M, Daniel SE, Quinn NP (1994) Clinical features and natural history of multiple system atrophy. An analysis of 100 cases. Brain 117(Pt 4):835–845

Wenning GK, Colosimo C, Geser F, Poewe W (2004a) Multiple system atrophy. Lancet Neurol 3(2):93–103

Wenning GK, Geser F, Krismer F, Seppi K, Duerr S, Boesch S, Kollensperger M, Goebel G, Pfeiffer KP, Barone P, Pellecchia MT, Quinn NP, Koukouni V, Fowler CJ, Schrag A, Mathias CJ, Giladi N, Gurevich T, Dupont E, Ostergaard K, Nilsson CF, Widner H, Oertel W, Eggert KM, Albanese A, del Sorbo F, Tolosa E, Cardozo A, Deuschl G, Hellriegel H, Klockgether T, Dodel R, Sampaio C, Coelho M, Djaldetti R, Melamed E, Gasser T, Kamm C, Meco G, Colosimo C, Rascol O, Meissner WG, Tison F, Poewe W (2013) The natural history of multiple system atrophy: a prospective European cohort study. Lancet Neurol 12(3):264–274

Wenning GK, Granata R, Laboyrie PM, Quinn NP, Jenner P, Marsden CD (1996) Reversal of behavioural abnormalities by fetal allografts in a novel rat model of striatonigral degeneration. Mov Disord 11(5):522–532

Wenning GK, Jellinger KA (2005) The role of alpha-synuclein in the pathogenesis of multiple system atrophy. Acta Neuropathol 109(2):129–140

Wenning GK, Krismer F, Poewe W (2011a) New insights into atypical parkinsonism. Curr Opin Neurol 24(4):331–338

Wenning GK, Litvan I, Tolosa E (2011b) Milestones in atypical and secondary Parkinsonisms. Mov Disord 26(6):1083–1095

Wenning GK, Stefanova N, Jellinger KA, Poewe W, Schlossmacher MG (2008) Multiple system atrophy: a primary oligodendrogliopathy. Ann Neurol 64(3):239–246

Wenning GK, Tison F, Ben Shlomo Y, Daniel SE, Quinn NP (1997) Multiple system atrophy: a review of 203 pathologically proven cases. Mov Disord 12(2):133–147

Wenning GK, Tison F, Seppi K, Sampaio C, Diem A, Yekhlef F, Ghorayeb I, Ory F, Galitzky M, Scaravilli T, Bozi M, Colosimo C, Gilman S, Shults CW, Quinn NP, Rascol O, Poewe W (2004b) Development and validation of the unified multiple system atrophy rating scale (UMSARS). Mov Disord 19(12):1391–1402

Wullner U, Schmitt I, Kammal M, Kretzschmar HA, Neumann M (2009) Definite multiple system atrophy in a German family. J Neurol Neurosurg Psychiatry 80(4):449–450

Yabe I, Soma H, Takei A, Fujiki N, Yanagihara T, Sasaki H (2006) MSA-C is the predominant clinical phenotype of MSA in Japan: analysis of 142 patients with probable MSA. J Neurol Sci 249(2):115–121

Yazawa I, Giasson BI, Sasaki R, Zhang B, Joyce S, Uryu K, Trojanowski JQ, Lee VM (2005) Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. Neuron 45(6):847–859

Youdim MB, Amit T, Falach-Yogev M, Bar Am O, Maruyama W, Naoi M (2003) The essentiality of Bcl-2, PKC and proteasome-ubiquitin complex activations in the neuroprotective-antiapoptotic action of the anti-Parkinson drug, rasagiline. Biochem Pharmacol 66(8):1635–1641

Yun JY, Lee WW, Lee JY, Kim HJ, Park SS, Jeon BS (2010) SNCA variants and multiple system atrophy. Ann Neurol 67(4):554–555

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Llorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yebenes JG (2004) The new mutation, E46 K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55(2):164–173

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