ASSESSMENT OF NOVEL THERAPEUTIC APPROACHES IN THE YAC128 MOUSE MODEL OF HUNTINGTON DISEASE

by

MAHMOUD A. POULADI

B.Sc., McMaster University 2001
M.Sc., McMaster University 2004

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

The Faculty of Graduate Studies

(Medical Genetics)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

September 2010

ABSTRACT

Huntington disease (HD) is a progressive disorder characterized by involuntary movements, emotional disturbances, and memory loss. The cardinal neuropathological feature of HD is loss of medium spiny neurons within the striatum. There is currently no cure for HD and the disease is ultimately fatal. Accumulating evidence has implicated excitotoxicity, a process in which excessive signaling via the glutamate receptors results in neurotoxicity, in the selective neuronal loss in HD. The main aim of the studies presented was to evaluate the potential of small molecule therapeutics known to target excitotoxicity-related pathways in the YAC128 transgenic mouse model of HD. We examined whether treatment with memantine, a clinically well-tolerated NMDA receptor antagonist currently used to treat patients with moderate to severe Alzheimer's disease, can improve the phenotype of YAC128 HD mice. We demonstrated that treatment with memantine results in improvements in motor function and rescues the striatal deficits in a dose-specific manner. Rasagiline is a selective inhibitor of monoamine oxidase type B (MAO-B) clinically approved for the treatment of Parkinson's disease that has been shown to protect against a number of neurotoxic stimuli. We demonstrate that treatment with rasagiline protects against striatal lesioning in acute models of excitotoxicity and improves the motor function of the YAC128 HD mice. We next examine in a qualitative manner whether treatment with a combination of memantine and rasagiline yields greater benefit than obtained with either compound alone. We demonstrate that treatment with a combination of memantine and rasagiline provides early and sustained improvements in motor function and rescues striatal deficits in the YAC128 HD mice. Induction of a heat shock protein (HSP) response has been shown to be neuroprotective in acute excitotoxicity models and in models of polyglutamine-

induced neurodegenerative disease. We also examined whether treatment with arimoclomol, a compound show to enhance the HSP response by prolonging the activation of heat shock factor 1 (Hsf-1), can improve the phenotype of the YAC128 HD mice. Our findings demonstrate that treatment with arimoclomol does not lead to up-regulation of an HSP response or rescue of the behavioural and striatal deficits in the YAC128 HD mice. Finally, we characterize psychiatric disturbances in YAC128 mice, demonstrating that YAC128 HD mice exhibit depressive-like symptoms as assessed by the Porsolt forced swim test and the sucrose consumption test of anhedonia. These measures that may be employed in assessing any anti-depressive effects of candidate treatments in preclinical therapeutic trials. Our findings suggest that targeting excitotoxicity may be a viable therapeutic approach in HD.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1 inositol (1,4,5)-triphosphate receptor (InsP3R1)

3-hydroxykynurenine (3-HK)

6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX)

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

Adenosine triphosphate (ATP)

Alzheimer's disease (AD)

Amino acids (aa)

Amyotrophic Lateral Sclerosis (ALS)

Analysis of Variance (ANOVA)

Arimoclomol (ARM)

Brain-derived neurotrophic factor (BDNF)

cAMP-response element binding protein (CREB)

cJun N-terminal kinase 3 (JNK3)

D-(-)-2-amino-5-phosphonovaleric acid (D-APV)

Diaminobenzidine (DAB)

Dopamine- and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32)

Embryonic stem (ES)

glutamate aspartate transporter (GLAST)

Glutamate transporter-1 (GLT-1)

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Heat shock factor 1 (Hsf-1)

Heat shock protein (HSP)

Histone acetyltransferase (HAT)

Histone deacetylase (HDAC)

Huntingtin (htt)

Huntingtin associated protein (HAP)

Huntington Disease (HD)

Hypothalamic-pituitary-adrenal (HPA)

Hypoxanthine phosphoribosyltransferase gene (Hprt)

Intraperitoneal (i.p.)

Kainate (KA)

Kilo basepair (kb)

Medium spiny neurons (MSNs)

Middle cerebral artery occlusion (MCAO)

Mitochondrial permeability transition (mPT)

Monoamine oxidase type B (MAO-B)

Mutant huntingtin (mtHtt)

N-methyl-D-aspartate (NMDA)

Neuron restrictive silencer element (NRSE)

Parkinson's disease (PD)

Phosphate buffered saline (PBS)

Polyglutamine (polyQ)

Porsolt forced swim test (FST)

Quinolinic acid (QA)

Repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF)

Revolutions per minute (rpm)

Selective serotonin reuptake inhibitor (SSRI)

Short hairpin RNA (shRNA)

Small interfering RNA (siRNA)

Sodium dodecyl sulfate (SDS)

Specificity protein 1 (Sp1)

spinocerebellar ataxia (SCA)

Standard error of the mean (SEM)

Stress-induced hyperthermia (SIH)

Wild-type (WT)

Wild-type huntingtin (wtHtt)

Yeast artificial chromosome (YAC)

Yellow fluorescent protein (YFP)

ACKNOWLEDGEMENTS

This dissertation is the culmination of a collaborative endeavor involving a great team of talented individuals to whom I owe much gratitude. I am greatly indebted to Michael Hayden for his supervision, mentorship, and friendship over the past 7 years. He has given me the opportunity to be involved in a myriad of experiences from which I have learned immensely. His tireless pursuit of knowledge, his commitment to science, and his humanitarian efforts have been a great source of inspiration, and have challenged me to be better and reach higher. I would also like to acknowledge the debt I owe to my supervisory committee members, Drs Yu Tian Wang, Jon Stoessl, and Lorne Clarke, and Dr Blair Leavitt for sharing their wisdom and valuable advice over the years, and Dr Stuart Lipton for an enjoyable and fruitful collaboration.

This work has also benefited immensely from interactions with other members of the lab and to them I would like to express my deep appreciation. In particular, I would like to thank Rona Graham, Yvonne Bombard, and Alicia Semaka for their great friendship, Jeff Carroll, Roshni Singaraja, and Liam Brunham for many fascinating discussions over the years, and Yuanyun Xie, Dagmar Ehrnhoefer, Nagat Bissada, Rachelle Dar Santos, Lisa Bertram, Malgorzata Zapala, Claudia Schwab, Sonia Franciosi, and members of the Hayden, Leavitt, and Raymond labs for generously sharing their technical advice and expertise.

Last, but not least, I would like to thank my parents, my brothers, and Susana for their unconditional love, encouragement, and support over the years. I am greatly indebted to them, and words cannot possibly describe my gratitude.

DEDICATION

To my parents for your endless

support, love, and encouragement

CO-AUTHORSHIP STATEMENT

Chapter 2

M.A.P. initiated and conducted the experiments, performed the data analysis, and contributed to manuscript writing. J.B.C., R.D.R., and L.N.B. assisted in conducting experiments. M.R.H. conceived and supervised the study and contributed to manuscript writing.

Chapter 3

S.-i.O. and D.Y. designed and performed the in vitro experiments. R.Z. and A.C. assisted with the in vitro experiments. M.K. offered key advice and helped analyze the in vitro experiments on mtHtt inclusions and cell death. M.A.P. and M.R.H. conceived the mouse studies. M.A.P. designed, conducted, and analyzed the mouse studies. D.E.E. and R.K.G. assisted in conducting the biochemical and neuropathology studies on mouse tissues. M.R.H. conceptualized and supervised the mouse studies. M.T. and P.X. performed the electrophysiology experiments. D.Z., H.-S.V.C., G.T. and S.A.L. supervised the electrophysiological experiments and gave crucial advice. S.-i.O., M.A.P., M.T., D.Y. and M.R.H. wrote the first draft of the manuscript. S.-i.O. and S.A.L. formulated the hypothesis and wrote the manuscript.

Chapter 4

M.A.P. conceived the study, initiated and performed the experiments, and contributed to manuscript writing. R.D.S. assisted with mouse behavioural studies. G.L. performed the stereotaxic intrastriatal injections. L.N.B. and M.Z. assisted in conducting the neuropathology histological assessments. B.R.L. made conceptual contributions to the study. M.R.H. supervised the study and contributed to manuscript writing.

Chapter 5

M.A.P. conceived the study, initiated and performed the experiments, and contributed to manuscript writing. R.D.S. and Y.X. assisted with mouse behavioural studies. M.Z. assisted in conducting the neuropathology histological assessments. M.R.H. conceived and supervised the study and contributed to manuscript writing.

1. Introduction

1.1. Historical background

Huntington disease bears the name of George Huntington who at the young age of 21 described the choreic condition and its hereditary nature (Hayden, 1981). His father and grandfather were both medical doctors, allowing an opportunity for his first encounter with the disease at the young age of 8 while accompanying his father on his professional rounds in East Hampton, NY (Hayden, 1981). This early exposure to the condition likely contributed to his great insight into the disease. Indeed, following publication of his thorough yet concise paper 'On Chorea' in 1872, his name later became associated with the condition in different parts of the world and has remained since then (Hayden, 1981).

1.2. Clinical manifestations of HD

1.2.1. Motor dysfunction

The major motor sign of HD is chorea, characterized by involuntary movements which are continuously present during waking hours, cannot be voluntarily suppressed by the patient, and worsen during stress. Chorea is a feature of HD in over 90% of patients, increasing during the first phase (~10 years) of the patients' illness and is seen less frequently in patients with juvenile onset (Folstein et al., 1986; Hayden, 1981). Additional motor deficits include oculomotor dysfunction (Hicks et al., 2008; Starr, 1967), gait disturbances, dysarthria (Podoll et al., 1988), dysphagia (Kagel and Leopold, 1992), bradykinesia, rigidity and dystonia (Hayden, 1981), which predominate in the late stages of the illness.

While the clinical diagnosis of disease onset in HD mutation carriers is generally defined on the basis of the appearance of motor dysfunction, other symptoms such as cognitive and psychiatric disturbances can precede deficits in motor coordination by many years.

1.2.2. Cognitive disturbances

Carriers of the HD mutation have cognitive deficits both prior to and following clinical diagnosis of disease onset. Presymptomatic HD carriers have deficits in strategy shifting, psychomotor speed, recognition memory, planning and verbal fluency (Berrios et al., 2002; Hahn-Barma et al., 1998; Lawrence et al., 1998a; Lawrence et al., 1998b; Paulsen et al., 2001; Snowden et al., 2002). HD patients have deficits in executive function (impaired ability to integrate new knowledge, slowness of thought, altered personality, and affective changes), impairments in memory (particularly visuospatial and procedural memory), and attention and concentration deficits (Ho et al., 2003; Pillon et al., 1991; Snowden et al., 2001; Witjes-Ané et al., 2003). While verbal memory remains mostly intact until the final stages of the illness, language learning is impaired early in HD patients (De Diego-Balaguer et al., 2008).

1.2.3. Psychiatric disturbances

While the symptomatic phase of HD is defined by the onset of motor symptoms, psychiatric disturbances usually present before the manifestation of motor symptoms. Mood and affective changes are indeed common, particularly depression which has been reported to occur in as many as 40% to 50% of HD patients (Duff et al., 2007; Heathfield, 1967; Kirkwood et al., 2001; Pflanz et al., 1991). Suicidal ideation and manic or hypomanic episodes are also more common in HD patients compared to the general population (Farrer, 1986; Folstein, 1991). Other psychiatric symptoms seen in HD patients include apathy, aggressive behavior, sexual

disinhibition, alcohol abuse, delusions, obsessions/compulsions, and psychosis (Burns et al., 1990).

1.2.4. Other manifestations

A number of manifestations outside the CNS have been identified in HD (van der Burg et al., 2009). For example, weight loss is frequently seen in HD patients (Aziz et al., 2008; Djoussé et al., 2002; Robbins et al., 2006; Sanberg et al., 1981), occurs despite increased appetite and caloric intake (Trejo et al., 2004), and is also seen in early stage patients exhibiting minimal chorea and thus cannot be accounted for by increased energy expenditure due to choreic movements (Goodman et al., 2008). Higher body mass index at diagnosis correlates with a lower rate of disease progression (Myers et al., 1991). There is also evidence for testicular degeneration and a reduction in plasma testosterone levels in male HD patients that correlate with disease severity (Markianos et al., 2005; Van Raamsdonk et al., 2007).

Other manifestations include sleep disturbances (Aziz et al., 2009; Emser et al., 1988; Wiegand et al., 1991a; Wiegand et al., 1991b), and incontinence, which occurs in approximately 20% of all patients in late phases of the illness (Hayden, 1981).

1.3. Neuropathology and neurochemistry

1.3.1. Neuropathology

The cardinal pathologic feature of HD is atrophy of the caudate nucleus and the putamen (the neostriatum), with the extent of neostriatal abnormalities correlating with the duration of the illness and severity of disease (Vonsattel et al., 1985).

On the macroscopic level, brains of HD patients generally appear atrophic, with reductions of as much as 400 grams in weight in advance cases of the disease (de la Monte et al., 1988). The atrophy is most severe in the caudate, followed by the putamen, and finally the nucleus

accumbens (Roos et al., 1986; Vonsattel et al., 1985). Cortical atrophy is also present with relative preservation of the normal layered architecture but with alterations in neocortical structures and layers III, V, and VI (de la Monte et al., 1988; Sotrel et al., 1991). The thalamus, and subthalamic nucleus may be reduced in size in late stages of disease, and in proportion to the rest of the brain (Lange et al., 1976). Severe atrophy in the hypothalamic lateral nucleus is also observed while other brain stem nuclei are generally spared (Averback, 1981; Clark et al., 1983; Kremer et al., 1990; Kremer et al., 1991; Rodda, 1981; Tagliavini and Pilleri, 1983). Grossly-evident atrophy of the cerebellum is rare except in juvenile cases or at the late phases of the illness (Rodda, 1981).

Microscopically, the neostriatal atrophy is typified by neuronal loss and gliosis (Vonsattel et al., 1985), with loss of medium and small sized neurons and relative sparing of larger interneurons (Lange et al., 1976). On the cellular level, multiple abnormalities in sub-cellular organelles including the nucleus and nucleolus, the endoplasmic reticulum, ribosomes, the Golgi apparatus, mitochondria, and lysosomes are present in neurons of the caudate nuclear in HD. Abnormal dendritic branching, elongation of distal dendrites, and alterations in spine densities in medium spiny neurons are also observed (Ferrante et al., 1991; Graveland et al., 1985). Astrocyte-type gliosis may also be present (Vonsattel et al., 1985).

1.3.2. Neurochemistry

Of the neuronal populations affected in HD, the GABAergic medium spiny neurons of the striatum that express enkephalin (Enk) or Substance P are the first and most affected (Kowall et al., 1987). Medium-sized aspiny neurons expressing nicotinamide adenine dinucleotide phosphate diaphorate (NADPH-d) and somatostatin or neuropeptide Y as well as large aspiny neurons expressing choline acetyltransferase (ChAT) are selectively spared in the striatum in HD

(Ferrante et al., 1985; Ferrante et al., 1987). Dopaminergic and serotonergic afferents are also spared (Beal et al., 1990; Spokes, 1980).

The primate neostriatum is organized into two regions: the striosomes and matrix compartments. This division is based on the amount of acetylcholinesterase (ACh) activity with striosomes showing weak staining for ACh while the surrounding matrix compartment shows more dense staining (Goldman-Rakic, 1982). The striosome-matrix organization is relatively preserved in HD (Vonsattel and Difiglia, 1998) with neuronal loss being observed in both compartments, initially in the striosomes and later in the matrix compartment (Hedreen and Folstein, 1995).

1.4. Genetics

Huntington disease is an autosomal dominant disorder. It was once thought to be fully penetrant, although it is now clear that the disease may not manifest within expected lifespan in a proportion of *HD* gene carriers (Brinkman et al., 1997; McNeil et al., 1997). While complete penetrance is observed for CAG size ≥ 42, only about 91% of those with CAG sizes of 36-41 manifest symptoms of HD within a normal lifespan (Brinkman et al., 1997).

1.4.1. The *HD* gene

In 1993, a novel gene containing a polymorphic CAG trinucleotide repeat sequence was identified in HD patients (Group, 1993). This CAG repeat, located in the 5' region of the Huntington gene, ranges from 10 to 35 copies on normal chromosomes, but is expanded beyond 36 repeats in HD patients, with expansions of over 200 CAG trinucleotides being observed (Kremer et al., 1994; Nance et al., 1999).

The human *HD* gene is located on chromosome 4p16.3 and, comprised of 67 exons encoding 3144 amino acids, spans 170 kb (Ambrose et al., 1994; Group, 1993). The *HD* gene is highly conserved, with a 90% homology between the human and the mouse genes and a 69% identity

between the human gene and the pufferfish homologue. Furthermore, a 100% conservation of the first 17 amino acids is seen across the human, mouse and Fugu proteins (Barnes et al., 1994; Baxendale et al., 1995; Lin et al., 1994; Schmitt et al., 1995).

1.4.2. Relationship between CAG repeat length and clinical manifestations

The number of CAG repeats has a strong inverse correlation with the age of onset of HD, ascertained on the basis of choreic movements (Andrew et al., 1993; Duyao et al., 1993; Nørremølle et al., 1993; Snell et al., 1993), with the number of CAG repeats in the expanded allele accounting for up to 70% of the variation in the age of onset (Brinkman et al., 1997; Langbehn et al., 2009; Langbehn et al., 2004). Although data from early studies did not allow for meaningful predictions for individuals to be made (Andrew et al., 1993; Duyao et al., 1993; Nørremølle et al., 1993; Snell et al., 1993), more recent studies which included carefully ascertained at-risk persons with CAG expansion who are presymptomatic showed that it is possible to use CAG size to broadly predict with broad confidence limits expected age of onset (Brinkman et al., 1997; Langbehn et al., 2009; Langbehn et al., 2004).

While there may be exceptions (Ahmad Aziz et al., 2009; Aziz et al., 2008), repeat length is generally not predictive of other clinical phenotypes. For example, there is no relationship between CAG repeat length and the prevalence or severity of depression in HD patients (Berrios et al., 2001; Craufurd et al., 2001; Kingma et al., 2008).

1.5. Excitotoxicity and HD

1.5.1. Background

A number of cellular aberrations have been delineated in HD since the identification of the mutation in 1993, including disruptions in gene transcription, cellular transport, and protein proteolysis (Roze et al., 2008; Wellington et al., 2000). However, even prior to the identification

of these alterations, excitotoxicity, a process in which excessive signaling via the glutamate receptors results in neurotoxicity, had been supported by substantial evidence as a key player in the pathogenesis in HD, and evidence accumulated since the identification of the mutation has provided further support in this regard (Fan and Raymond, 2007).

Indeed, the involvement of excitotoxicity in the pathogenesis of HD was first suggested by rodent studies in which intrastriatal injections of kainate (KA) or quinolinic acid (QA), an endogenous metabolic product of the kynurenine pathway, produced lesions that mimicked many of the neurochemical and histopathological features of HD (Beal et al., 1986; Beal et al., 1988; Coyle and Schwarcz, 1976; DiFiglia, 1990; McGeer and McGeer, 1976) and were associated with HD-like behavioural deficits (Beal et al., 1991a; Furtado and Mazurek, 1996; Popoli et al., 1994). A number of human and animal studies have since identified defects in NMDA and mGluR5 receptor signaling, as well as mitochondrial calcium handling in HD patients and animal models of HD. Collectively, these studies give rise to a coherent, multifactorial model of mutant huntingtin-mediated alteration of glutamate receptor activity and calcium signaling as a primary contributor to neuronal degeneration in HD (Shear et al., 1998).

1.5.2. Role of NMDA receptors

Involvement of NMDA receptors in the pathology of HD was initially inferred from two lines of studies. The first set of studies demonstrated that injection of glutamate agonists into the striatum of rodents results in HD-like neuronal lesions. For example, injection of kainate into the striatum of rats was found to induce HD-like neuropathological changes. This effect of kainate was dependent on glutamate release as it was prevented by prior decortication and removal of the corticostriatal afferents (Biziere and Coyle, 1978; Biziere and Coyle, 1979). Further studies showed that injection of the NMDA receptor agonist QA into the striatum of rodents resulted in

the most accurate reproduction of the histological and neuropathological changes seen in HD (Beal et al., 1986; DiFiglia, 1990), and was associated with HD-like behavioural changes in lesioned animals as well (Beal et al., 1991a; Furtado and Mazurek, 1996; Popoli et al., 1994). The second set of studies showed that neurons expressing NMDA receptors seem to be preferentially lost in HD, suggesting a role for NMDA receptors in enhancing the susceptibility to cell death. For example, analysis of post-mortem brain tissues from patients with HD showed that NMDA receptor binding was reduced by 93 percent in the putamen from HD brains compared to binding in normal brains (Young et al., 1988). Furthermore, in situ hybridization histochemistry studies of rat striatum showed that striatal projection neurons, the population selectively lost in HD patients, displayed enhanced expression of NR1/NR2B-type NMDA receptors compared to the spared interneurons (Landwehrmeyer et al., 1995). The difference in NMDA receptor subtype expression was suggested to contribute to the relative vulnerability and resistance of striatal projection and interneurons, respectively, to NMDA receptor-mediated excitotoxicity.

While these studies demonstrated the capacity of NMDA receptor overactivation to cause HD-like neuropathological and behavioural changes and provided correlative evidence from brains of HD patients, no evidence of a direct modulation of NMDA receptor function by mutant huntingtin was provided. Using huntingtin and NMDA receptor co-transfected HEK293 cells, the first such evidence demonstrated that mutant (Htt-138Q), but not wildtype (Htt-15Q), huntingtin enhances NMDA receptor currents (Chen et al., 1999), an effect that is specific for the NR1/NR2B NMDA receptor subtype and not the NR1/NR2A subtype. Using the same co-transfection system, it was subsequently shown that mutant huntingtin (Htt-138Q) leads to increased susceptibility to NMDA receptor-mediated cell death compared to wildtype huntingtin

(Htt-15Q) (Zeron et al., 2001). Significantly, this increase in NMDA receptor-mediated excitotoxic death is markedly diminished when an N-terminal fragment of mutant huntingtin is used in place of the full-length mutant protein. Furthermore, the enhancement of NMDA receptor-mediated cell death by mutant huntingtin is greater in cells transfected with the NR1/NR2B NMDA receptor subtype compared to the NR1/NR2A subtype. That the potentiation of NMDA-induced currents and enhancement of sensitivity to NMDA receptor-mediated cell death by mutant huntingtin are NR2B-specific is intellectually satisfying as NR1/NR2B is the principal NMDA receptor subtype expressed in medium spiny neurons of the striatum (Landwehrmeyer et al., 1995).

These observations of enhanced NMDA receptor activity in the presence of mutant huntingtin were further validated in the YAC72 transgenic mouse model of HD. The YAC72 transgenic mice express the entire human HD gene with 72 CAG repeats under the control of the endogenous huntingtin promoter and regulatory elements, and recapitulate many of the behavioural and neuropathological features of the human condition (Hodgson et al., 1999). Using whole-cell patch clamp recordings, it was demonstrated that NMDA receptor peak current amplitudes and current density are significantly larger in medium spiny neurons from YAC72 mice compared to wildtype (Zeron et al., 2002). It was further demonstrated that medium spiny neurons from YAC72 mice show enhanced susceptibility to QA and NMDA-induced cell death compared to wildtype. Similar findings demonstrating enhanced striatal susceptibility to QA and NMDA-mediated excitotoxicity were made in the transgenic YAC mice expressing the entire human HD gene with 128 CAG repeats (YAC128 mice) (Tang et al., 2005; Graham et al., 2006). Furthermore, this enhancement is specific to medium spiny neurons and is not observed in cerebellar granule neurons from YAC72 mice (Zeron et al., 2002), an observation consistent with

the pathology of HD in which no apparent cerebellar degeneration is observed. Corroborating the subtype-specificity reported in HEK293 co-transfection studies, treatment of medium spiny neurons from YAC72 with ifenprodil, an NR2B-specific NMDA receptor antagonist, prevents excitotoxic cell death, further implicating the NR1/NR2B NMDA receptor subtype in HD.

The enhanced excitotoxic cell death mediated by NMDA receptors was shown to occur via the intrinsic apoptotic pathway using primary medium spiny neuronal cultures from YAC46 and YAC72 mice (Zeron et al., 2004), an observation that was also validated using primary medium spiny neurons isolated from YAC128 animals (Shehadeh et al., 2006; Tang et al., 2005). Furthermore, while defects in mitochondrial function were shown to contribute to the enhancement in NMDA receptor-mediated cell death, the difference in the extent to which mitochondrial stressors alone enhance cell death compared to NMDA receptor-mediated cell death indicates that NMDA receptor function and/or NMDA receptor-specific downstream signaling partners are also altered by mutant huntingtin (Shehadeh et al., 2006).

1.5.3. Synaptic versus extrasynaptic NMDA receptors

Although NMDA receptors have been implicated in excitotoxicity and neuronal death, they play an important physiological role in mediating synaptic transmission and influencing neuronal development. Indeed, inhibition or elimination of NMDA receptor activity results in widespread neuronal abnormalities and apoptosis in developing brains (Adams et al., 2004; Gould et al., 1994; Monti and Contestabile, 2000; Pohl et al., 1999) and exacerbates trauma-induced neuronal loss in adulthood (Ikonomidou et al., 1999). Similar effects of NMDA receptor activity and blockade on cellular survival have been reproduced in cultured neuronal models as well (Hardingham et al., 2002; Hardingham and Bading, 2003; Hetman and Kharebava, 2006), and have been shown to be mediated via the PI3K-Akt pathway and CREB-mediated gene

expression (Hardingham et al., 2002; Hetman and Kharebava, 2006; Lafon-Cazal et al., 2002; Papadia et al., 2005) as well as the induction of antioxidant defense machinery (Papadia et al., 2008). Thus, an episode of NMDA receptor activation can either boost neuronal health and promote survival or result in the initiation of an apoptotic cascade that culminates in neuronal demise. Whether NMDA receptor activation results in neuronal survival or death depends at least partly on two primary parameters. First, as alluded to in the previous section, the magnitude of receptor activation which is determined by the intensity and duration of activation, is a major determinant of the outcome of NMDA receptor activity, with excessive (or insufficient) activation leading to neuronal death. Second, the location of NMDA receptor activation is also an important parameter. Whereas synaptic NMDA receptor activity mediates pro-survival signals, such as CREB-dependent gene expression (Hardingham et al., 2002; Zhang et al., 2007), activation of extrasynaptic NMDA receptors shuts off pro-survival pathways and promotes neuronal death (Hardingham et al., 2002; Zhang et al., 2007).

Whether an NMDA receptor activation episode results in survival or death is therefore dependent on both the magnitude of the activation and the location of the receptor, parameters that underlie the bell shape of the NMDA receptor activity-neuronal survival curve (Figure 1.1).

Recent studies have elegantly demonstrated that extrasynaptic NMDA receptor expression and currents are significantly increased in transgenic YAC HD mice before onset of disease phenotype, are associated with reductions in nuclear CREB activation, and correlate with CAG length (Milnerwood et al., 2010). These findings implicate increased extrasynaptic NMDA receptor activity in disease pathology, and support targeting excitotoxicity as a therapeutic approach in HD.

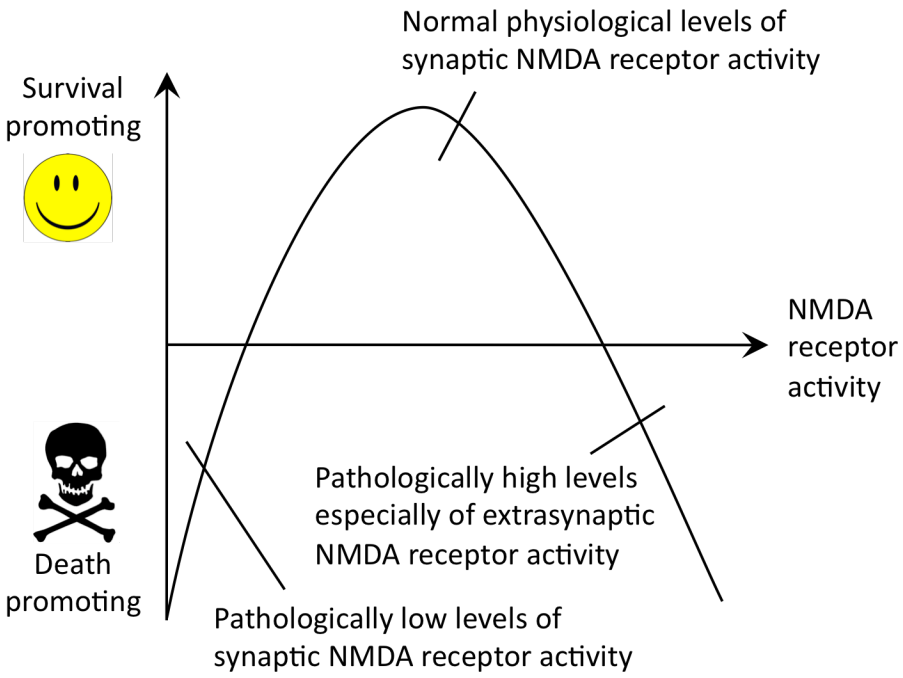


Figure 1.1 The dichotomous nature of NMDA receptor activity.

NMDA receptor activity can have both pro-survival and pro-death effects depending on the magnitude as well as the location of the activity. Low levels of synaptic NMDA receptor activity result in failure to induce sufficient pro-survival pathways leading to neuronal death. High levels of activity, primarily by extrasynaptic NMDA receptors, lead to inhibition of pro-survival pathways, such as CREB-mediated transcription, and induction of mitochondrial depolarization and apoptotic neuronal death. Adapted from Hardingham and Bading, Trends in Neurosciences, 2003, 26 (2): 81-9 with permission.

1.5.4. Involvement of metabolites of the kynurenine pathway

Further evidence in support of excitotoxicity as key pathogenic process in HD comes from studies examining the levels of endogenous metabolites known to influence the excitotoxic pathway. Indeed, levels of the NMDA receptor agonist QA are increased in the cortex and striatum of YAC128 HD animals (Guidetti et al., 2006). Furthermore, levels of another metabolic product of the kynurenine pathway known to potentiate QA-mediated excitotoxicity, 3-hydroxykynurenine (3-HK), are elevated in the cortex and striatum of YAC128 HD animals (Guidetti et al., 2006). Similar increases in QA and 3-HK levels have been detected in the neocortex and neostriatum of early grade HD brains (Guidetti et al., 2004), and the increases in 3-HK and QA are of the same order of magnitude as those known to induce neuron loss in vitro (Chiarugi et al., 2001; Okuda et al., 1996; Whetsell and Schwarcz, 1989). In light of the role of QA and 3-HK in mediating excitotoxicity, these changes in their endogenous levels in both the brains of HD patients and YAC128 HD mice lend support to excitotoxicity as a pathogenic mechanism contributing to HD.

1.5.5. Role of mitochondria

The involvement of mitochondria in neurodegenerative diseases in general and HD in particular has been long recognized. Clues about the involvement of the mitochondria in HD were provided by studies showing compromised energy metabolism in the form of decrease glucose utilization or increased lactate concentrations which preceded bulk neuronal loss in the caudate and putamen of HD patients (Grafton et al., 1990; Hayden et al., 1986; Jenkins et al., 1993; Kuhl et al., 1982; Kuwert et al., 1993; Mazziotta et al., 1987). Similar defects in energy metabolism were also seen the cerebral cortex and muscle tissue of HD patients (Koroshetz et al., 1997). Several subsequent studies identified striatal-specific mitochondrial defects in post-mortem brains

(Browne et al., 1997; Gu et al., 1996; Tabrizi et al., 1999) and peripheral tissues of HD patients (Arenas et al., 1998; Brennan et al., 1985; Browne et al., 1997; Gu et al., 1996; Mann et al., 1990; Panov et al., 2002; Sawa et al., 1999; Tabrizi et al., 1999). Experimental studies in animals provided further support for the involvement of the mitochondria in HD. Treatment of rodents and non-human primates with mitochondrial toxins led to neuronal degeneration that mimicked neurodegenerative changes observed in human HD (Beal et al., 1993a; Brouillet et al., 1993; Brouillet et al., 1995). Mitochondria from lymphoblasts of HD patients had a lower membrane potential, depolarized more readily and to a greater extent following challenge compared to normal individuals (Panov et al., 2002; Sawa et al., 1999). Enhanced sensitivity to mitochondrial toxins is similarly seen in clonal striatal neurons derived the *Hdh*Q111 knock-in HD mice (Mao et al., 2006; Ruan et al., 2004). Furthermore, mitochondria from brains of transgenic YAC72 HD mice are less resistant to calcium challenge than mitochondria from brain of wildtype or YAC18 control animals (Panov et al., 2002). This has the effect of activating the mitochondrial permeability transition and causing the release of calcium and apoptotic factors, effectively facilitating calcium dysregulation and the induction of cell death.

More direct evidence for a mitochondrial role in potentiating NMDA receptor-mediated excitotoxicity was demonstrated in transgenic YAC models of HD. Using primary medium spiny neurons from YAC46 mice, it was demonstrated that inhibition of the mitochondrial permeability transition with cyclosporine A, bongkrekic acid, or boosting mitochondrial function with coenzyme Q10, substantially diminishes NMDA receptor-mediated cell death (Zeron et al., 2004) and abolishes the observed difference in NMDA receptor-mediated cell death between YAC46 and wildtype. Similar results were obtained from medium spiny neurons from YAC128 mice (Fernandes et al., 2007; Tang et al., 2005). These findings also highlight mitochondrial and

excitotoxicity-related cellular disturbances as early events likely to contribute to disease pathogenesis.

The mitochondrial defects in HD brains and peripheral tissues likely reflect a direct effect of mutant htt as indeed it is found to associate directly with mitochondria (Choo et al., 2004; Gutekunst et al., 1998; Panov et al., 2002). For example, it was suggested that mutant htt may influence mitochondrial calcium handling directly by forming ion channels in the mitochondrial membrane (Panov et al., 2002). Alternatively, mutant htt may influence mitochondrial fission and fusion by interfering with the cellular machinery involved in these processes, and lead to the altered mitochondrial properties observed (Bossy-Wetzel et al., 2008). Treatment of cortical neurons with 3-NP, a mitochondrial toxin known to produced HD-like neurochemical changes when administered to rodents and primates (Brouillet et al., 1993; Brouillet et al., 1995), results in mitochondrial fission and cell death that is abolished by treatment with an NMDA receptor antagonist, suggesting that energy deficits in HD can facilitate cell death via NMDA receptor-dependent secondary excitotoxicity (Liot et al., 2009). Furthermore, increased mitochondrial fragmentation in the presence of oxidative stress is seen in HeLa cells expressing mutant htt fragments, and treatments that inhibit of mitochondrial fission or promote mitochondrial fusion prevent this enhanced fragmentation in mutant htt-expressing cells and the associated cell death observed (Wang et al., 2009).

1.6. Huntingtin function

Huntingtin is a 350kDa multi-domain protein that is thought to have a role in development, transcriptional regulation, cellular trafficking, and neuroprotection. The protein is expressed throughout the body and is found predominantly in the cytoplasm within cells, although it has been also been detected in the nucleus (Cattaneo et al., 2005).

1.6.1. Development

Huntingtin appears to play a critical role in embryonic development. Indeed, targeted disruption of exon 1 (Zeitlin et al., 1995), exon 5 (Nasir et al., 1995), or exons 4 and 5 (Duyao et al., 1995) of *Hdh*, the murine homologue of the HD gene, results in post-implantation embryonic lethality approximately at embryonic day 8.5, shortly after the induction of gastrulation. Injection of *Hdh* null ES cells into WT host blastocysts, but not vice versa, rescues the embryonic lethality, suggesting that the importance of htt during embryonic development involves extraembryonic tissues, such as the visceral endoderm, and their physiological functions such as providing nutrients to the developing embryo (Dragatsis et al., 1998), and not necessarily cell autonomous effect within embryonic tissues. In support of this notion, while htt was shown to be required for normal differentiation of ES cells into hematopoietic cells (Metzler et al., 2000), *Hdh* null ES cells were shown to differentiate into mature, post-mitotic neurons expressing functional voltage- and neurotransmitter-gated ion channel with functional synapses (Metzler et al., 1999). Furthermore, *Hdh* null neurons in chimeric animals generated by injecting *Hdh* null ES cells into WT blastocysts differentiate and are found throughout the brain in adult animals (Reiner et al., 2001).

Expansion of the CAG repeat tract within the *HD* gene does not appear to disrupt its role in embryogenesis and development. Patients homozygous for the *HD* mutation have normal development (Dürr et al., 1999; Squitieri et al., 2003), and homozygous knock-in mice expressing *Hdh* with a CAG repeat length of 50 are viable and show no developmental abnormalities (White et al., 1997). Furthermore, transgenic YAC mice expression mutant htt with 72 CAG repeats can rescue the embryonic lethality of *Hdh* null mice (Leavitt et al., 2001). These studies indicate that the function of htt in embryogenesis and development is maintained in the presence of the polyglutamine expansion.

Interestingly, the importance of htt during development does not appear to extend to invertebrates. In Drosophila in which the drosophila homologue of the *HD* gene (*dhtt*) has been inactivated develop normally with no obvious defects (Zhang et al., 2009). This, along with the observation that no *HD* gene homologue is found in *C. elegans* (Li et al., 1999), suggests that htt does not play an important role in the development of invertebrates, which may reflect intrinsic differences in the embryogenesis of vertebrates and invertebrates (Zhang et al., 2009).

1.6.2. Neuroprotection

Huntingtin has been shown to have anti-apoptotic and neuroprotective properties. Over-expression of htt in clonal striatal cells provided protection against a range of apoptotic stimuli (Rigamonti et al., 2001). Similar effects were seen in neuroblastoma cells where over-expression of htt protected against the neurotoxicity mediated by the N-terminal fragment of htt (Ho et al., 2001).

In vivo, htt has been shown to provide partial rescue of the testicular degeneration induced by mutant htt (Leavitt et al., 2001) and to be neuroprotective against excitotoxic neuronal death brought about by systemic or intrastriatal injection of excitatory agents (Leavitt et al., 2006; Zhang et al., 2006). This pro-survival function of htt has been shown to be associated with decreased activation (Leavitt et al., 2006; Rigamonti et al., 2000) or direct inhibition of caspase-3 (Zhang et al., 2006), but may also reflect the role htt plays in the expression of neuroprotective factors, such as brain-derived neurotrophic factor (BDNF) (Zuccato et al., 2001).

1.6.3. Transcription

A potential role for huntingtin in transcriptional regulation was initially suggested by studies showing altered levels mRNA transcripts, particularly those coding for neurotransmitter receptors, in post-mortem brains of HD patients (Augood et al., 1996; Augood et al., 1997).

Similar changes in mRNA transcript levels were observed in mouse models of HD and, importantly, preceded neuronal loss and onset of symptoms, indicating that such changes do not simply reflect loss of selective neuronal populations but may in fact contribute to the disease pathology (Cha et al., 1999). Subsequent gene expression profiling studies have shown widespread transcriptional dysregulation in cellular and mouse models of HD (Chan et al., 2002; Luthi-Carter et al., 2000; Luthi-Carter et al., 2002a; Luthi-Carter et al., 2002b; Sipione et al., 2002). Specific interactions between htt and a number of transcriptional factors have since been identified (reviewed in (Kazantsev and Hersch, 2007; Sugars and Rubinsztein, 2003)). For example, htt has been shown to regulate the expression of BDNF (Zuccato et al., 2001) by interacting with and sequestering repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF), an effect that is impaired by the polyglutamine expansion in mutant htt (Zuccato et al., 2003). Htt has also been shown to influence the transcriptional activity of the transcription factors Sp1, TAFII130, CREB, and PGC-1α (Cui et al., 2006; Dunah et al., 2002; Obrietan and Hoyt, 2004; Weydt et al., 2006). These findings were confirmed using an in vitro transcriptional assay where the factors Sp1, TFIID, and TFIIF were found to be direct targets inhibited by mutant htt in a polyglutamine-dependent manner (Zhai et al., 2005). Furthermore, a recent study implicates htt in the regulation of the transcriptional activity of liver X receptors, an effect that is impaired in mutant htt (Futter et al., 2009).

In addition to its association with and regulation of transcription factor activity, htt has been shown to decrease histone acetyltransferase (HAT) activity in neuronal cells expressing mutant htt (Igarashi et al., 2003). This influence on HAT activity is associated with hypo-acetylation in both cellular and animal models of HD (Igarashi et al., 2003; Sadri-Vakili et al., 2007) and likely contributes to the transcriptional dysregulation and resultant disease pathology observed. Indeed,

treatment with inhibitors of histone deacetylase (HDAC) corrects the reported hypo-acetylation (Sadri-Vakili et al., 2007) and rescues the disease phenotype in animal models of HD (Pallos et al., 2008; Steffan et al., 2001; Thomas et al., 2008).

1.6.4. Intracellular trafficking

Early studies demonstrating the predominant cytoplasmic localization of htt and its association with vesicles suggested a role for htt in vesicle trafficking (Difiglia et al., 1995). Further studies showed that htt associates with microtubules (Hoffner et al., 2002; Tukamoto et al., 1997) and endocytic proteins (Velier et al., 1998), and interacts with proteins involved in vesicle trafficking and intracellular transport such as HAP1 (Block-Galarza et al., 1997; Li et al., 1998; Li et al., 1995), HIP1 (Kalchman et al., 1997; Metzler et al., 2001; Wanker et al., 1997), optineurin (Sahlender et al., 2005), and dynein (Caviston et al., 2007). Polyglutamine expansion in htt has been shown to impair its role in intracellular trafficking and is thought to contribute to the pathology in HD. Indeed, mutant htt has been shown to inhibit axonal transport in isolated squid axoplasm (Szebenyi et al., 2003), Drosophila (Gunawardena et al., 2003; Lee et al., 2004), and mouse and human HD brains (Trushina et al., 2004), with such defects being paralleled by impairment in trafficking of cellular organelles such as mitochondria (Trushina et al., 2004). These impairments in axonal transport by mutant htt have been attributed to aggregates formed by mutant htt (Chang et al., 2006), and more recently to activation of neuron-specific cJun N-terminal kinase 3 (JNK3) and phosphorylation of the motor domain of kinesin-1 (Morfini et al., 2009). Furthermore, htt has been implicated in post-Golgi trafficking along both secretory (Strehlow et al., 2007) and lysosomal (del Toro et al., 2009) paths.

Of particular importance to the pathogenesis of HD is the demonstrated role of htt in the vesicular transport of BDNF along microtubules and its impairment by mutant htt (del Toro et

al., 2006; Gauthier et al., 2004). The observed decrease in striatal BDNF levels may not only represent impaired transcription (Zuccato et al., 2003), but also disrupted endocytosis of cortically-secreted BDNF along the corticostriatal neuronal circuit (Gauthier et al., 2004).

1.7. Huntingtin inclusions and HD

1.7.1. Inclusions and neurotoxicity

A feature of neurodegenerative diseases, and particularly trinucleotide repeat disorders, is the presence of insoluble protein aggregates, also known as inclusions (Scherzinger et al., 1997; Schulz and Dichgans, 1999). Initial studies in post-mortem human HD brains showed that inclusions are present in the affected areas of the brain, namely the cortex and striatum (Difiglia et al., 1997), and increased in size with disease progression (Gutekunst et al., 1999). That similar inclusions were observed in transgenic HD mice, with the formation of these inclusions taking place prior to the onset of neurological symptoms was interpreted to suggest a pathogenic role for inclusions in HD (Bates, 2003; Davies et al., 1997; Morton et al., 2000). A number of studies have since aimed to reduce inclusion load as a therapeutic strategy (Colby et al., 2004; Desai et al., 2006; Huang et al., 2007; Sánchez et al., 2003).

While these studies suggest a potential pathogenic contribution of inclusions to HD, other observations cast doubt on such a role (reviewed in (Slow et al., 2006)). Indeed, studies of post-mortem HD brains show that only 1-4% of the neurons in the most affect region of the brain, the striatum, contain inclusions, in contrast to the less affected cortex where there are more widespread inclusions (Gutekunst et al., 1999). In several animal models of HD, inclusions appear many months after the onset of motor and cognitive deficits (Menalled et al., 2003; Slow et al., 2003; Van Raamsdonk et al., 2005), and neuronal loss (Slow et al., 2003). In animal models where inclusions do precede the onset of the phenotype, such as the R6/2,

pharmacological and genetic approaches that result in improvement of the phenotype either have no effect on inclusions (Ferrante et al., 2003; Hockly et al., 2003), or in fact increase the inclusion load (Mastroberardino et al., 2002). In wildtype-R6/2 chimeric mice, genotypically R6/2 neurons survive for more than 10 months, far longer than the 15-week lifespan of the pure R6/2 animals, despite containing inclusions that are similar both in size and frequency to those found in pure R6/2 animals (Reiner et al., 2007). Further, in a full-length mutant htt cDNA mouse model where behavioural abnormalities and striatal degeneration are observed, inclusions are only seen in a very small percentage of cells and are present in regions and cellular populations that are largely spared from pathology in HD (Reddy et al., 1999). Furthermore, in a transgenic YAC mouse termed shortstop expressing a fragment of htt with identical polyglutamine length, tissue distribution, and level of expression as the full-length YAC128 HD model, inclusions are detected earlier, are more frequent, and have greater tissue distribution than seen in the YAC128 HD mice (Slow et al., 2005). Despite this, shortstop mice do not exhibit the motor dysfunction or neuronal loss observed in YAC128 mice (Slow et al., 2005). These observations collectively demonstrate that HD-related phenotypic abnormalities can be present in the absence of inclusions and that inclusions can be present in the absence of behavioural and neuronal deficits.

Other studies have in fact pointed to a protective role for inclusions in HD. In neurons transfected with mutant htt that were monitored over time, those that formed inclusions had an increased likelihood of survival compared to those that did not (Arrasate et al., 2004). This protective property of inclusions was proposed to reflect their function as ‘sinks’ sequestering the toxic soluble forms of mutant htt, as indeed the levels of diffuse mutant htt were decreased in neurons that formed inclusions (Arrasate et al., 2004). Pharmacological strategies to promote htt

inclusion formation have recently been pursued as a therapeutic approach in HD (Bodner et al., 2006).

1.7.2. Formation of huntingtin inclusions

The formation of inclusions in HD has been shown to involve molecular chaperones, such as Hsp70/Hsp40, that bind to non-native proteins and aid in their folding or facilitate their degradation (Muchowski, 2002). Recently, other components of the cellular chaperone machinery have been shown to influence htt inclusion formation (Behrends et al., 2006; Kitamura et al., 2006; Tam et al., 2006). A genome-wide RNA interference screen in *C. elegans* employing yellow fluorescent protein (YFP)-polyglutamine fusion proteins to study inclusion formation in the presence of polyglutamine expansion implicated, in addition to Hsp70 and Hsp40, 6 of the 8 components of the molecular chaperon TRiC (Nollen et al., 2004). TRiC assists in the folding of *de novo* proteins partly by facilitating the actions of Hsp70 (Frydman, 2001; Hartl and Hayer-Hartl, 2002). Recent studies have demonstrated a role for TRiC in modulating the formation of htt inclusions, by promoting the formation of non-toxic high-molecular weight htt oligomers (Behrends et al., 2006). In particular, over-expression of the CCT1 subunit of TRiC was shown to increase htt inclusions and decrease toxicity in neurons in vitro (Tam et al., 2006). These findings suggest that factors that influence TRiC expression or activity levels may in turn influence htt inclusion formation and toxicity in HD.

1.8. Experimental models of HD

1.8.1. Neurotoxin models

A number of neurotoxin-based models of HD have been described over the years. These models were developed following initial observations that injection of kainic acid, a glutamate receptor agonist, produced striatal lesioning of GABAergic projection neurons while sparing striatal

afferents (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Subsequent studies showed that quinolinic acid, an NMDA receptor agonist, produced excitotoxic striatal lesions that more faithfully recapitulated the neurochemical changes seen in HD brains (Beal et al., 1986; Beal et al., 1991b; Ferrante et al., 1993). Intrastriatal injections of excitatory agonists, particularly QA, have since been used in rodents and primates as an acute model of HD.

A second group of neurotoxin-based models of HD employ mitochondrial toxins to induce acute striatal lesions. This class of neurotoxin comprises mitochondrial inhibitors, such as malonate and 3-nitropropionic acid (3-NP), which target complexes of the electron transport chain, thereby reducing ATP levels and causing cellular energy depletion (Beal et al., 1993a; Brouillet et al., 1993). Reduced ATP levels result partial membrane depolarization and removal of the voltage-dependent Mg^{2+} block of NMDA receptors, leading to NMDA receptor-dependent secondary excitotoxicity and striatal lesioning (Novelli et al., 1988; Zeevalk and Nicklas, 1991). These mitochondrial toxins have also been used in both rodents and primates as acute models of HD (Beal et al., 1993b; Brouillet et al., 1995; Ferrante et al., 1993; Henshaw et al., 1994).

1.8.2. Mouse models

Since the cloning of the *HD* gene in 1993, a number of mouse and rat models have been generated. Only mouse models of HD will be briefly described here. They vary in several aspects including the promoter used, the length and level of the htt protein, the source of the htt protein (human versus mouse), as well as the CAG repeat length, but can be broadly classified into two categories: 1) mice expressing a truncated N-terminal fragment of mutant htt, and 2) mice expressing the full-length mutant htt.

1.8.2.1. N-terminal fragment mouse models

A number of transgenic mouse models expressing a fragment of htt have been generated. These models typically express a truncated N-terminal fragment under the control of either a non-endogenous promoter (Laforet et al., 2001; Schilling et al., 1999; Yamamoto et al., 2000) or only a short fragment of the endogenous htt promoter (Mangiarini et al., 1996). The resulting htt protein fragments range in size from 67 amino acids representing exon 1 in the R6/2 (Mangiarini et al., 1996) and the conditional HD94 Yamamoto and Hen mice (Yamamoto et al., 2000), 171 amino acids in the N171-82Q mice (Schilling et al., 1999), to about 1000 amino acids in the HD47 and HD100 mice (Laforet et al., 2001).

Animals expressing a truncated N-terminal fragment of htt typically exhibit an early onset of symptoms, including motor dysfunction, behavioural abnormalities, weight loss, and reduced survival (Mangiarini et al., 1996; Schilling et al., 1999; Yamamoto et al., 2000). Furthermore, these symptoms are often paralleled by widespread inclusions and a generalized, non-selective neurodegenerative phenotype in the CNS (Mangiarini et al., 1996; Schilling et al., 1999; Yamamoto et al., 2000). Given this rapid onset of symptoms in these truncated fragment models of HD, the animals have been widely used in preclinical therapeutic trials (reviewed in (Beal and Ferrante, 2004; Gil and Rego, 2009)). However, the validity of these models and their predictive power as preclinical tools in therapeutic endeavors is unclear. Indeed, animals expressing a variant of hypoxanthine phosphoribosyltransferase gene (Hprt) in which 146 CAG (Hprt-CAG146) repeats have been introduced into exon 3 developed a neurological phenotype similar to that of N-terminal fragment models of HD, exhibiting motor abnormalities along with widespread inclusions and reduced lifespan (Ordway et al., 1997). The findings from the Hprt-CAG146 study along with the fact that the HD mutation in N-terminal fragment models lacks the protein context of the full-length protein and the proper promoter and regulatory sequences,

suggests that the neurological phenotype observed in these fragment models of HD likely reflects generalized polyglutamine toxicity and may not necessarily reflect HD-specific phenotypes.

1.8.2.2. Full-length mouse models

Several mouse models expressing full-length mutant htt were generated either by introducing a CAG repeat expansion into the endogenous mouse *Hdh* gene (knock-in full-length mice), or by transgenically expressing the human mutant htt gene (transgenic full-length mice).

The knock-in mice generated carry mutant *Hdh* genes with CAG repeats sizes ranging from 50 to 150 (Heng et al., 2007; Lin et al., 2001; Menalled et al., 2002; Shelbourne et al., 1999; Wheeler et al., 2000; White et al., 1997). These mice represent genetically accurate models, carrying one copy of mutant htt and one copy of wildtype htt at the endogenous levels with temporally and spatially appropriate expression pattern of htt. Characterization of knock-in mice has revealed neurological phenotype in the majority of these mouse models, although the symptoms are mostly mild and are paralleled with either little or no neurodegeneration (Lin et al., 2001; Menalled et al., 2002; Menalled et al., 2003; Shelbourne et al., 1999; Wheeler et al., 2000; White et al., 1997). An exception is the *Hdh*-CAG150 knock-in mice, which show motor deficits starting at 18 months of age, and decreases in striatal volume and neuronal counts at 25, but not 18 months of age; paradoxically, however, the striatal volume deficits are only seen in homozygous but not heterozygous *Hdh*-CAG150 mice as would be expected in a dominantly-inherited disease (Heng et al., 2007). Thus, while representing genetically accurate models of HD and reproducing some of the phenotypes of human HD, the mild phenotype of these models and protracted time-course over which they develop limits their usefulness for proof-of-concept therapeutic studies.

Transgenic mice expressing the full-length human genomic *HD* gene with intact introns and exons have been generated using yeast and bacterial artificial chromosome technology (YAC and BAC transgenesis) (Gray et al., 2008; Hodgson et al., 1999; Slow et al., 2003). Transgenic YAC mice expressing mutant htt with 18, 47, 72, and 128 CAG repeat lengths have been generated (Hodgson et al., 1999; Slow et al., 2003). In addition to the full-length *HD* gene, these constructs include 25-kb upstream and 120-kb downstream regulatory sequences and as such yield appropriate temporal and tissue-specific expression of mutant htt. Of the full-length BAC and YAC models, the YAC128 HD mice are the most characterized and recapitulate several features of human HD. These mice exhibit age-dependent selective striatal and subsequently cortical neurodegeneration. Cognitive deficits are observed starting at 2 months of age (Van Raamsdonk et al., 2005), and progressive motor dysfunction is evident by 3 months of age, which is highly correlated with neuronal loss in the striatum (Lerch et al., 2008; Slow et al., 2003). The phenotype of the YAC128 HD mice is in contrast to the mild and delayed phenotype observed in knock-in full-length mouse models of HD, and may reflect differences in htt expression levels, strain background effects, or species-specific sequence differences, such as miRNA target sequences, between human mutant htt expressed in the transgenic mice and murine mutant htt expressed in the knock-in mice (reviewed in (Ehrnhoefer et al., 2009)). Thus, the presence of the full-length protein context, the appropriate tissue-specific and temporal expression of mutant htt, along with the robust and early behavioural abnormalities and selective neurodegenerative phenotypes in the YAC128 animals make this mouse model of HD particularly suited for trial of therapeutic candidates.

1.9. Neuroprotective treatments for HD

Despite tremendous progress in our understanding of the mechanisms underlying HD, all clinical treatments currently available are aimed at providing symptomatic relief to patients. Despite several large-scale trials in HD patients, no treatment has thus far been shown to prevent or significantly delay the onset or rate of progression of the disease.

1.9.1. Translation of basic findings to approaches to therapies

The advent of animals models of HD has not only allowed investigations into the disrupted cellular processes that underlie the disease but has also enabled the interrogation of these processes as therapeutic targets in HD. Trial of therapeutic candidates aimed at modulating a range of targets, including transcriptional dysregulation (Ferrante et al., 2003; Ferrante et al., 2004; Hockly et al., 2003; Steffan et al., 2001), aggregation (Tanaka et al., 2004; Wood et al., 2007), oxidative stress (Andreassen et al., 2001; Klivenyi et al., 2003), energy deficits (Ferrante et al., 2002; Schilling et al., 2001), apoptosis (Chen et al., 2000; Ona et al., 1999), and excitotoxicity (Ferrante et al., 2002; Hockly et al., 2006; Schilling et al., 2001) have been conducted in animal models of HD.

Of these candidates, those that have been taken forward into clinical trials have had limited success. This apparent failure in translating therapeutic success from animal models to patients likely reflects a number of factors. For example, although several cellular abnormalities and disrupted pathways have been identified, the natural order of their occurrence and their primacy in the evolution of the disease remains unclear. In the case of a linear progression, disruption of one pathway is an obligatory precedent for another cellular dysfunction; for example, increased NMDAR activity and the accompanying calcium influx lead to increased demand for ATP production, resulting in an increase in oxidative phosphorylation and reactive oxygen species

production, which in turn cause mitochondrial damage and eventual permeability transition pore opening and excitotoxicity-related apoptotic cell death (Figure 1.2). Under such circumstances, targeting early and initiating focal events may suffice to fully ameliorate the disease, whereas targeting downstream cellular disruptions may only lead to partial benefit.

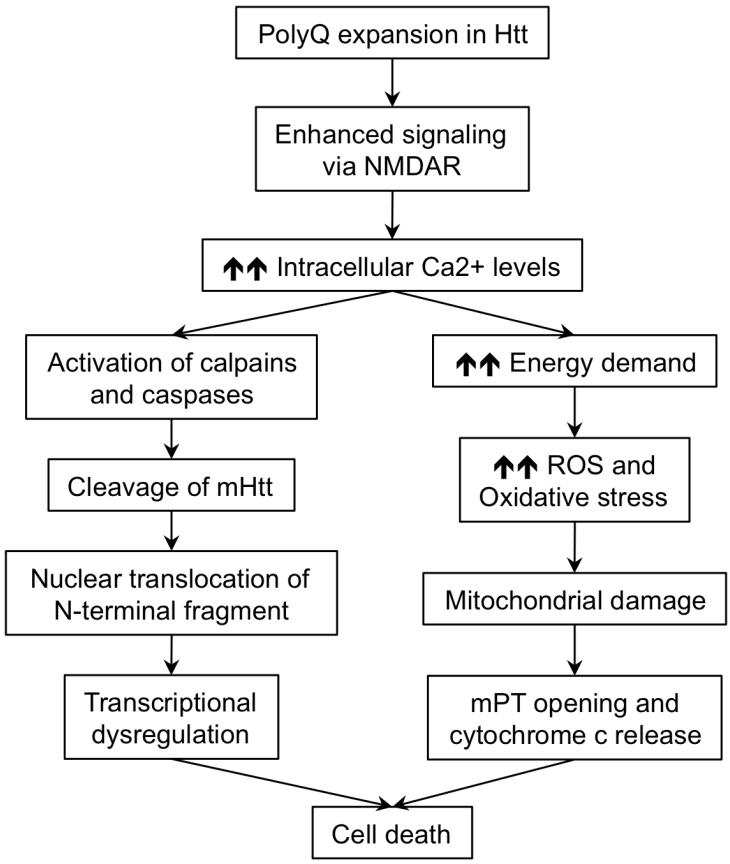


Figure 1.2 A model of linear progression of pathogenic processes leading to Huntington disease.

Under such linear progression model, early and initiating focal events may suffice to ameliorate the disease.

An alternative and likely scenario is the simultaneous disruption of multiple cellular pathways by mutant htt (Figure 1.3). Under such a model, targeting any one pathway in isolation, as has been the case in the majority of trials conducted thus far, may only result in partial benefit. If this is the case, the failure of these treatments in human trials makes it likely that the targeted pathways represent downstream and not initiating events in the disease pathogenesis. Another likely reason behind the poor translation of therapeutic success from animal models to patients is the timing of the treatment. A luxury afforded by animal models is the ability to treat subjects pre-symptomatically and before the onset of manifestation that often signal significant cellular dysfunction and demise. Intervention at this early stage likely improves the chances of slowing the neuronal loss and preserving the integrity of the neural circuitry, a practice that has been difficult to adopt in human trials thus far.

Nonetheless, therapeutic trials in animal models and HD patients should reflect the advances made in understanding the biology of the disease and accompanying targets identified for intervention, as well as the availability of improved or better suited pharmacological and biological reagents to engage these targets. Failure of a given treatment to improve symptoms or ameliorate the disease may indicate either that the target is not suitable for therapeutic intervention, or that the treatment chosen to modulate it is not optimal. Indeed, in addition to choice of target, the "what to treat", other parameters such as when to treat (symptomatically or pre-symptomatically), how much to treat with (dose), and for how long (the duration) all can influence the success of the treatment. Treatments aimed at modulating excitotoxicity in HD are illustrative in this regard.

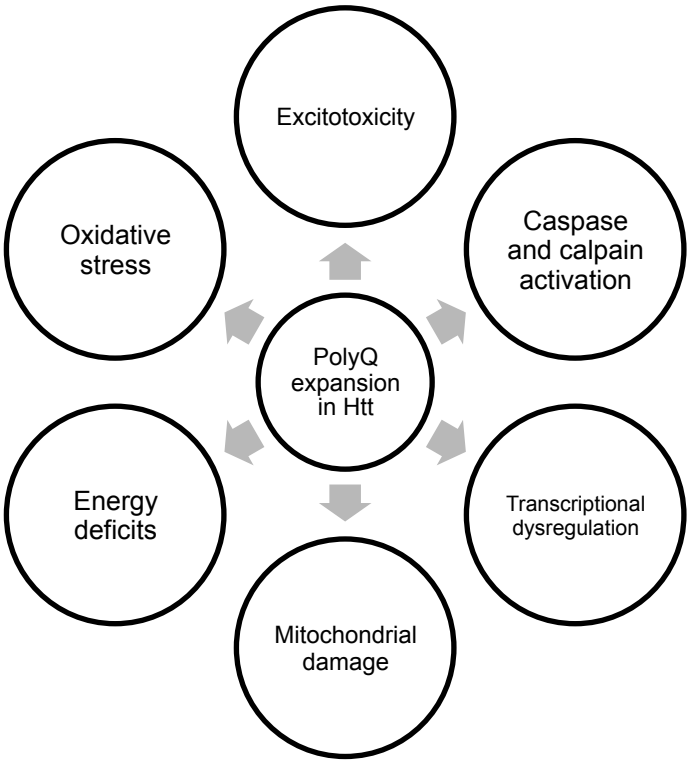


Figure 1.3 A model of simultaneous pathogenic disruption of multiple cellular pathways leading Huntington disease

Under such a multiple cellular disruption model, targeting several disrupted pathways may be necessary to prevent of delay the progression of the disease.

1.9.2. Trials of compounds targeting excitotoxicity in HD

Examinations of the enhanced excitotoxicity in cellular and rodent models of HD demonstrate its presence as an early phenotype, well before the appearance of the behavioural or motor deficits that are characteristic of HD. Interventions aimed to combating the enhanced susceptibility to excitotoxic stress are, therefore, more likely to succeed if administered to gene carriers at a pre-symptomatic stage before disease manifestation. However, the slow progressing nature of the illness, coupled with the lack of sensitive longitudinal diagnostic tests that are able to measure disease progression in pre-symptomatic patients, has presented a major challenge to trial of such anti-excitotoxic interventions.

Excitotoxicity has long been implicated in HD, and evidence from acute toxin-based and genetic animal models of HD as well as HD patients strongly supports its involvement (see Section 1.5). As such, several compounds with anti-excitotoxic properties have been evaluated in HD patients (Table 1-1). Several single-treatment doses of ketamine, an NMDAR antagonist commonly used in anesthesia, were evaluated in 10 symptomatic HD patients and compared to placebo treatments on a second identical testing day (Murman et al., 1997). This acute treatment did not result in any improvement in any of the parameters measured (Murman et al., 1997), which may relate to the high level of inhibition of NMDARs whose activity is essential for proper CNS function or the extremely short duration of treatment.

Table 1-1 Summary of Clinical Trials of Anti-Excitotoxicity Drugs in HD

Drug	Target	Type of trial	Doses	Duration of trial	Disease duration	No. of patients	Outcome	Ref.
Riluzole	Anti-XTC; Glutamate uptake	Open-label	50 mg, b.i.d.	6 wk	6 yr	8	Positive	(Rosas et al., 1999)
		OL	50 mg, b.i.d.	12 m	8 yr	9	Positive	(Seppi et al., 2001)
		DB-PC	100 mg, 200 mg	8 wk	5-6 yr	56	Positive (at 200 mg)	(Group, 2003)
		DB-PC	50 mg, b.i.d.	3 yr	5-6 yr	379	Negative	(Landwehrmeyer et al., 2007)
Lamotrigine	Glutamate release	DB-PC	400 mg	2.5 yr	3 yr	55	Negative (symptomatic improvement reported)	(Kremer et al., 1999)
Ketamine	NMDAR	DB-PC	0.10, 0.40, and 0.60 mg/kg/hr	Single infusion	4.7 yr	10	Negative	(Murman et al., 1997)
Remacemide	NMDAR	DB-PC	200 mg, t.i.d.	2.5 yr	5 yr	173	Negative	(Group, 2001)
Amantadine	Anti-XTC	OL	100 mg, or 200 mg	n/a	n/a	6	Positive	(Gray et al., 1975)
		OL	100 mg, t.i.d.	1 yr	5 yr	8	Positive	(Lucetti et al., 2002)
		DB-PC	400 mg	2 wk	6 yr	22	Positive	(Verhagen Metman et al., 2002)
		DB-PC	200 mg IV, 100 mg t.i.d. orally	2-hr for IV, 1 yr for oral	5 yr	9	Positive	(Lucetti et al., 2003)
		DB-PC	300 mg	6 wk	6 yr	8	Positive	(Heckmann et al., 2004)
Memantine	NMDAR	OL	≤ 30 mg	2 yr	2.5 yr	27	Positive	(Beister et al., 2004)
		OL	20 mg	12 wk	–	9	Positive	(Ondo et al., 2007)

Abbreviations: DB-PC: Double-Blind, Placebo-Controlled; OL

Another drug with putative anti-excitotoxic properties is riluzole. Although its exact target is unclear (Mary et al., 1995), riluzole has been shown to protect against excitotoxicity in a number of toxin-based animal studies (Guyot et al., 1997; Mary et al., 1995; Palfi et al., 1997) and has been evaluated in open-label (Rosas et al., 1999; Seppi et al., 2001) and placebo-controlled trials (Group, 2003; Landwehrmeyer et al., 2007; Squitieri et al., 2008) in HD patients. In the open-label studies, treatment of patients with an average disease duration of 6-8 years resulted in significant improvements in motor and functional capacity scores compared to baseline following short-term (1.5 to 3 months) but not long-term treatment (12 months) (Rosas et al., 1999; Seppi et al., 2001). As a prelude to large multi-center trial with riluzole, a dose-ranging placebo-controlled study was conducted and found that treatment for 2 months of patients with average disease duration of 5-6 years significantly improved the total maximal chorea score compared to placebo (Group, 2003). A subsequent large, multi-center trial in which patients with average disease duration of 5-6 years were treated with riluzole or placebo for 3 years showed no neuroprotective or symptomatic benefits of riluzole treatment. Not withstanding a small recent MRI-based study suggesting a neuroprotective effect of riluzole on the basis of decreased gray matter loss and fluorodeoxyglucose uptake deficits in patients treated with riluzole (Squitieri et al., 2008), the results of the open-label and placebo-controlled trials suggest that riluzole treatment provides choreic improvements in the short-term with no neuroprotective or symptomatic relief benefits with long-term treatment.

Similarly, 30-month long placebo-controlled trials with lamotrigine, an anti-epileptic drug thought to protect against excitotoxicity by moderating pre-synaptic glutamate release, and remacemide, a noncompetitive NMDAR antagonist, showed no improvements in total functional

capacity in treated patients compared to placebo-treated controls (Group, 2001; Kremer et al., 1999), although a trend towards improved chorea was reported in the case of lamotrigine (Kremer et al., 1999). Furthermore, although no retardation of disease progression was observed, a greater proportion of lamotrigine-treated patients reported symptomatic improvement compared to placebo-treated patients (Kremer et al., 1999).

Amantadine is an anti-Parkinsonian (Danielczyk, 1995) and antiviral drug (Jefferson et al., 2001) that is also an uncompetitive NMDA receptor antagonist (Stoof et al., 1992a; Stoof et al., 1992b). Amantadine has been tested in open-label and placebo-controlled trials in symptomatic HD patients (Gray et al., 1975; Heckmann et al., 2004; Lucetti et al., 2002; Verhagen Metman et al., 2002). Short-term trials that were 2-week to 1-year long showed significant improvements in chorea (Gray et al., 1975; Lucetti et al., 2002; Verhagen Metman et al., 2002) and behaviour scores (Heckmann, 2004) in patients treated with amantadine. A placebo-controlled trial in which treatment was infused intravenously for a 2-hr period showed similar improvements in choreic movements, indicating that the effect of amantadine in HD patients likely reflects symptomatic relief and not disease modifying effects of amantadine (Lucetti et al., 2003).

Finally, two open-label trials of memantine, an uncompetitive NMDA receptor antagonist, have been conducted in HD patients. In a 4-month trial in 9 symptomatic HD, treatment with memantine significantly improved motor performance at the conclusion of the study compared to baseline (Ondo et al., 2007). In the second open-label trial, 24 HD patients with average disease duration of 30 months were treated with memantine and followed for 24 months (Beister et al., 2004). Assessment at 12 and 24 months showed no deterioration in psychometric tests or choreic movements, which were superior in comparison to what would be expected based on historic controls. Decreased scores were reported in two of the measured parameters, the “clinical global

impression" and "Huntington's disease activities of daily living", although these decreases were due to deteriorations in the first year, with no significant differences in the scores of these parameters between the assessments conducted at 12 months and 24 months (Beister et al., 2004). Furthermore, that no deterioration was observed between the 12 months and 24 months assessments suggests a possible neuroprotective effect of memantine requiring long-term treatment (Beister et al., 2004).

Overall, these anti-excitotoxic drugs appear to result in improvements in motor function in the short-term likely due to symptomatic action of the drugs but no long-term benefits. A common feature of these trials is that they involve symptomatic subjects. As a significant proportion of the neurons vulnerable in HD is lost prior to onset of the disease, the efficacy of anti-excitotoxic therapies in preserving neurons in symptomatic patients may be limited as the majority of the hyper-sensitive neurons in such patients would have been lost before the initiation of treatment. In this regard, inclusion of pre-symptomatic patients would provide a better setting in which to evaluate the neuroprotective effect of candidate anti-excitotoxic drugs. Furthermore, these studies indicate that special care should be given to the dose of drug to be administered, as acute improvements that are observed at given doses may represent symptomatic action and continued treatment at such doses may in fact lead to detrimental effects due to prolong or excessive action at a physiologically important target.

Thus, the trials conducted so far do not rule out a potential neuroprotective role for therapeutic candidates aimed at modulating excitotoxicity and indeed, the open-label memantine trial showing retardation in progression of disease over a 2-year period is supportive of this notion.

1.10. Objectives

The overall objective of this thesis was to evaluate the potential of excitotoxicity as a therapeutic target in HD using pharmacological agents in the transgenic YAC128 mouse model of HD. Previous studies have established that YAC128 HD animals recapitulate features of the human condition, exhibiting progressive motor and cognitive dysfunction and age-dependent selective striatal pathology. In this thesis, the specific objectives were as follows:

a. To examine whether, in addition to the motor and cognitive dysfunction, the YAC128 HD animals also exhibit psychiatric disturbances with the aim of developing psychiatric endpoints for use in therapeutic trials in these animals,

b. To evaluate the potential of heat shock proteins as a targeted in HD using the compound arimoclomol,

c. To evaluate NMDA receptors as a target in HD with the anti-Alzheimer's disease NMDA receptor antagonist memantine,

d. To evaluate mitochondrial permeability transition and pore opening as targets in HD using the anti-Parkinson's disease drug rasagiline, and

e. To examine the therapeutic efficacy of treatment with the compounds memantine and rasagiline in combination.

1.11. References

Adams, S.M., de Rivero Vaccari, J.C., Corriveau, R.A., 2004. Pronounced cell death in the absence of NMDA receptors in the developing somatosensory thalamus. J Neurosci. 24, 9441-50.

Ahmad Aziz, N., Pijl, H., Frölich, M., Maurits van der Graaf, A., Roelfsema, F., Roos, R., 2009. Leptin secretion rate increases with higher CAG repeat number in Huntington's disease patients. Clin Endocrinol (Oxf).

Ambrose, C.M., Duyao, M.P., Barnes, G., Bates, G.P., Lin, C.S., Srinidhi, J., Baxendale, S., Hummerich, H., Lehrach, H., Altherr, M., 1994. Structure and expression of the Huntington's disease gene: evidence against simple inactivation due to an expanded CAG repeat. Somat Cell Mol Genet. 20, 27-38.

Andreassen, O.A., Ferrante, R.J., Dedeoglu, A., Beal, M.F., 2001. Lipoic acid improves survival in transgenic mouse models of Huntington's disease. Neuroreport. 12, 3371-3.

Andrew, S.E., Goldberg, Y.P., Kremer, B., Telenius, H., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M.A., Graham, R.K., Hayden, M.R., 1993. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet. 4, 398-403.

Arenas, J., Campos, Y., Ribacoba, R., Martín, M.A., Rubio, J.C., Ablanedo, P., Cabello, A., 1998. Complex I defect in muscle from patients with Huntington's disease. Ann Neurol. 43, 397-400.

Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R., Finkbeiner, S., 2004. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature. 431, 805-10.

Augood, S.J., Faull, R.L., Love, D.R., Emson, P.C., 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, 1023-36.

Augood, S.J., Faull, R.L., Emson, P.C., 1997. Dopamine D1 and D2 receptor gene expression in the striatum in Huntington's disease. Ann Neurol. 42, 215-21.

Averback, P., 1981. Lesions of the nucleus ansae peduncularis in neuropsychiatric disease. Arch Neurol. 38, 230-5.

Aziz, N., Pijl, H., Frölich, M., Schröder-van der Elst, J., van der Bent, C., Roelfsema, F., Roos, R., 2009. Delayed onset of the diurnal melatonin rise in patients with Huntington's disease. J Neurol.

Aziz, N.A., van der Burg, J.M.M., Landwehrmeyer, G.B., Brundin, P., Stijnen, T., Group, E.S., Roos, R.A.C., 2008. Weight loss in Huntington disease increases with higher CAG repeat number. Neurology. 71, 1506-13.

Barnes, G.T., Duyao, M.P., Ambrose, C.M., McNeil, S., Persichetti, F., Srinidhi, J., Gusella, J.F., MacDonald, M.E., 1994. Mouse Huntington's disease gene homolog (Hdh). Somat Cell Mol Genet. 20, 87-97.

Bates, G., 2003. Huntingtin aggregation and toxicity in Huntington's disease. Lancet. 361, 1642-4.

Baxendale, S., Abdulla, S., Elgar, G., Buck, D., Berks, M., Micklem, G., Durbin, R., Bates, G., Brenner, S., Beck, S., 1995. Comparative sequence analysis of the human and pufferfish Huntington's disease genes. Nat Genet. 10, 67-76.

Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J., Martin, J.B., 1986. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 321, 168-71.

Beal, M.F., Kowall, N.W., Swartz, K.J., Ferrante, R.J., Martin, J.B., 1988. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J Neurosci. 8, 3901-8.

Beal, M.F., Matson, W.R., Swartz, K.J., Gamache, P.H., Bird, E.D., 1990. Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. J Neurochem. 55, 1327-39.

Beal, M.F., Ferrante, R.J., Swartz, K.J., Kowall, N.W., 1991a. Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. J Neurosci. 11, 1649-59.

Beal, M.F., Swartz, K.J., Finn, S.F., Mazurek, M.F., Kowall, N.W., 1991b. Neurochemical characterization of excitotoxin lesions in the cerebral cortex. J Neurosci. 11, 147-58.

Beal, M.F., Brouillet, E., Jenkins, B., Henshaw, R., Rosen, B., Hyman, B.T., 1993a. Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. J Neurochem. 61, 1147-50.

Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R., Hyman, B.T., 1993b. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci. 13, 4181-92.

Beal, M.F., Ferrante, R.J., 2004. Experimental therapeutics in transgenic mouse models of Huntington's disease. Nat Rev Neurosci. 5, 373-84.

Behrends, C., Langer, C.A., Boteva, R., Böttcher, U.M., Stemp, M.J., Schaffar, G., Rao, B.V., Giese, A., Kretzschmar, H., Siegers, K., Hartl, F.U., 2006. Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. Mol Cell. 23, 887-97.

Beister, A., Kraus, P., Kuhn, W., Dose, M., Weindl, A., Gerlach, M., 2004. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. J Neural Transm Suppl. 117-22.

Berrios, G.E., Wagle, A.C., Marková, I.S., Wagle, S.A., Ho, L.W., Rubinsztein, D.C., Whittaker, J., Ffrench-Constant, C., Kershaw, A., Rosser, A., Bak, T., Hodges, J.R., 2001. Psychiatric

symptoms and CAG repeats in neurologically asymptomatic Huntington's disease gene carriers. Psychiatry Res. 102, 217-25.

Berrios, G.E., Wagle, A.C., Marková, I.S., Wagle, S.A., Rosser, A., Hodges, J.R., 2002. Psychiatric symptoms in neurologically asymptomatic Huntington's disease gene carriers: a comparison with gene negative at risk subjects. Acta psychiatrica Scandinavica. 105, 224-30.

Biziere, K., Coyle, J.T., 1978. Influence of cortico-striatal afferents on striatal kainic acid neurotoxicity. Neuroscience Letters. 8, 303-10.

Biziere, K., Coyle, J.T., 1979. Effects of cortical ablation on the neurotoxicity and receptor binding of kainic acid in striatum. J. Neurosci. Res. 4, 383-98.

Block-Galarza, J., Chase, K.O., Sapp, E., Vaughn, K.T., Vallee, R.B., Difiglia, M., Aronin, N., 1997. Fast transport and retrograde movement of huntingtin and HAP 1 in axons. Neuroreport. 8, 2247-51.

Bodner, R.A., Outeiro, T.F., Altmann, S., Maxwell, M.M., Cho, S.H., Hyman, B.T., McLean, P.J., Young, A.B., Housman, D.E., Kazantsev, A.G., 2006. Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. Proc Natl Acad Sci USA. 103, 4246-51.

Bossy-Wetzel, E., Petrilli, A., Knott, A.B., 2008. Mutant huntingtin and mitochondrial dysfunction. Trends Neurosci. 31, 609-16.

Brennan, W.A., Bird, E.D., Aprille, J.R., 1985. Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem. 44, 1948-50.

Brinkman, R.R., Mezei, M.M., Theilmann, J., Almqvist, E., Hayden, M.R., 1997. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. Am J Hum Genet. 60, 1202-10.

Brouillet, E., Jenkins, B.G., Hyman, B.T., Ferrante, R.J., Kowall, N.W., Srivastava, R., Roy, D.S., Rosen, B.R., Beal, M.F., 1993. Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. J Neurochem. 60, 356-9.

Brouillet, E., Hantraye, P., Ferrante, R.J., Dolan, R., Leroy-Willig, A., Kowall, N.W., Beal, M.F., 1995. Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci USA. 92, 7105-9.

Browne, S.E., Bowling, A.C., MacGarvey, U., Baik, M.J., Berger, S.C., Muqit, M.M., Bird, E.D., Beal, M.F., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. 41, 646-53.

Burns, A., Folstein, S., Brandt, J., Folstein, M., 1990. Clinical assessment of irritability, aggression, and apathy in Huntington and Alzheimer disease. J Nerv Ment Dis. 178, 20-6.

Cattaneo, E., Zuccato, C., Tartari, M., 2005. Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci. 6, 919-30.

Caviston, J.P., Ross, J.L., Antony, S.M., Tokito, M., Holzbaur, E.L.F., 2007. Huntingtin facilitates dynein/dynactin-mediated vesicle transport. Proc Natl Acad Sci USA. 104, 10045-50.

Cha, J.H., Frey, A.S., Alsdorf, S.A., Kerner, J.A., Kosinski, C.M., Mangiarini, L., Penney, J.B., Davies, S.W., Bates, G.P., Young, A.B., 1999. Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. Philos Trans R Soc Lond, B, Biol Sci. 354, 981-9.

Chan, E.Y.W., Luthi-Carter, R., Strand, A., Solano, S.M., Hanson, S.A., DeJohn, M.M., Kooperberg, C., Chase, K.O., DiFiglia, M., Young, A.B., Leavitt, B.R., Cha, J.-H.J., Aronin, N., Hayden, M.R., Olson, J.M., 2002. Increased huntingtin protein length reduces the number of polyglutamine-induced gene expression changes in mouse models of Huntington's disease. Hum Mol Genet. 11, 1939-51.

Chang, D.T.W., Rintoul, G.L., Pandipati, S., Reynolds, I.J., 2006. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. Neurobiology of Disease. 22, 388-400.

Chen, M., Ona, V.O., Li, M., Ferrante, R.J., Fink, K.B., Zhu, S., Bian, J., Guo, L., Farrell, L.A., Hersch, S.M., Hobbs, W., Vonsattel, J.P., Cha, J.H., Friedlander, R.M., 2000. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med. 6, 797-801.

Chen, N., Luo, T., Wellington, C., Metzler, M., McCutcheon, K., Hayden, M.R., Raymond, L.A., 1999. Subtype-specific enhancement of NMDA receptor currents by mutant huntingtin. J Neurochem. 72, 1890-8.

Chiarugi, A., Meli, E., Moroni, F., 2001. Similarities and differences in the neuronal death processes activated by 3OH-kynurenine and quinolinic acid. J Neurochem. 77, 1310-8.

Choo, Y.S., Johnson, G.V.W., MacDonald, M., Detloff, P.J., Lesort, M., 2004. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet. 13, 1407-20.

Clark, A.W., Parhad, I.M., Folstein, S.E., Whitehouse, P.J., Hedreen, J.C., Price, D.L., Chase, G.A., 1983. The nucleus basalis in Huntington's disease. Neurology. 33, 1262-7.

Colby, D.W., Chu, Y., Cassady, J.P., Duennwald, M., Zazulak, H., Webster, J.M., Messer, A., Lindquist, S., Ingram, V.M., Wittrup, K.D., 2004. Potent inhibition of huntingtin aggregation and cytotoxicity by a disulfide bond-free single-domain intracellular antibody. Proc Natl Acad Sci USA. 101, 17616-21.

Coyle, J.T., Schwarcz, R., 1976. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature. 263, 244-6.

Craufurd, D., Thompson, J.C., Snowden, J.S., 2001. Behavioral changes in Huntington Disease. Neuropsychiatry, neuropsychology, and behavioral neurology. 14, 219-26.

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C.N., Tanese, N., Krainc, D., 2006. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 127, 59-69.

Danielczyk, W., 1995. Twenty-five years of amantadine therapy in Parkinson's disease. J Neural Transm Suppl. 46, 399-405.

Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L., Bates, G.P., 1997. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 90, 537-48.

De Diego-Balaguer, R., Couette, M., Dolbeau, G., Dürr, A., Youssov, K., Bachoud-Lévi, A.-C., 2008. Striatal degeneration impairs language learning: evidence from Huntington's disease. Brain. 131, 2870-81.

de la Monte, S.M., Vonsattel, J.P., Richardson, E.P., 1988. Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. J Neuropathol Exp Neurol. 47, 516-25.

del Toro, D., Canals, J.M., Ginés, S., Kojima, M., Egea, G., Alberch, J., 2006. Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. J Neurosci. 26, 12748-57.

del Toro, D., Alberch, J., Lázaro-Diéguez, F., Martín-Ibáñez, R., Xifró, X., Egea, G., Canals, J.M., 2009. Mutant huntingtin impairs post-Golgi trafficking to lysosomes by delocalizing optineurin/Rab8 complex from the Golgi apparatus. Mol Biol Cell. 20, 1478-92.

Desai, U.A., Pallos, J., Ma, A.A.K., Stockwell, B.R., Thompson, L.M., Marsh, J.L., Diamond, M.I., 2006. Biologically active molecules that reduce polyglutamine aggregation and toxicity. Hum Mol Genet. 15, 2114-24.

DiFiglia, M., 1990. Excitotoxic injury of the neostriatum: a model for Huntington's disease. Trends Neurosci. 13, 286-9.

Difiglia, M., Sapp, E., Chase, K., Schwarz, C., Meloni, A., Young, C., Martin, E., Vonsattel, J.P., Carraway, R., Reeves, S.A., 1995. Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron. 14, 1075-81.

Difiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P., Aronin, N., 1997. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science. 277, 1990-3.

Djoussé, L., Knowlton, B., Cupples, L.A., Marder, K., Shoulson, I., Myers, R.H., 2002. Weight loss in early stage of Huntington's disease. Neurology. 59, 1325-30.

Dragatsis, I., Efstratiadis, A., Zeitlin, S., 1998. Mouse mutant embryos lacking huntingtin are rescued from lethality by wild-type extraembryonic tissues. Development. 125, 1529-39.

Duff, K., Paulsen, J.S., Beglinger, L.J., Langbehn, D.R., Stout, J.C., Group, P.-H.I.o.t.H.S., 2007. Psychiatric symptoms in Huntington's disease before diagnosis: the predict-HD study. Biol Psychiatry. 62, 1341-6.

Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.-M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N., Krainc, D., 2002. Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science. 296, 2238-43.

Dürr, A., Hahn-Barma, V., Brice, A., Pêcheux, C., Dodé, C., Feingold, J., 1999. Homozygosity in Huntington's disease. J Med Genet. 36, 172-3.

Duyao, M., Ambrose, C., Myers, R., Novelletto, A., Persichetti, F., Frontali, M., Folstein, S., Ross, C., Franz, M., Abbott, M., 1993. Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet. 4, 387-92.

Duyao, M., Auerbach, A., Ryan, A., Persichetti, F., Barnes, G., McNeil, S., Ge, P., Vonsattel, J.-P., Gusella, J., Joyner, A., MacDonald, M., 1995. Inactivation of the Mouse Huntington's Disease Gene Homolog Hdh. Science. 269, 407-410.

Ehrnhoefer, D.E., Butland, S.L., Pouladi, M.A., Hayden, M.R., 2009. Mouse models of Huntington disease: variations on a theme. Dis Model Mech. 2, 123-9.

Emser, W., Brenner, M., Stober, T., Schimrigk, K., 1988. Changes in nocturnal sleep in Huntington's and Parkinson's disease. J Neurol. 235, 177-9.

Fan, M.M.Y., Raymond, L.A., 2007. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. Prog Neurobiol. 81, 272-93.

Farrer, L.A., 1986. Suicide and attempted suicide in Huntington disease: implications for preclinical testing of persons at risk. Am J Med Genet. 24, 305-11.

Fernandes, H.B., Baimbridge, K.G., Church, J., Hayden, M.R., Raymond, L.A., 2007. Mitochondrial sensitivity and altered calcium handling underlie enhanced NMDA-induced apoptosis in YAC128 model of Huntington's disease. J Neurosci. 27, 13614-23.

Ferrante, R., Kowali, N., Beal, M., Richardson, E., Bird, E., Martin, J., 1985. Selective Sparing of a Class of Striatal Neurons in Huntington's Disease. Science. 230, 561-563.

Ferrante, R.J., Beal, M.F., Kowall, N.W., Richardson, E.P., Martin, J.B., 1987. Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. Brain Res. 411, 162-6.

Ferrante, R.J., Kowall, N.W., Richardson, E.P., 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, 3877-87.

Ferrante, R.J., Kowall, N.W., Cipolloni, P.B., Storey, E., Beal, M.F., 1993. Excitotoxin lesions in primates as a model for Huntington's disease: histopathologic and neurochemical characterization. Experimental Neurology. 119, 46-71.

Ferrante, R.J., Andreassen, O.A., Dedeoglu, A., Ferrante, K.L., Jenkins, B.G., Hersch, S.M., Beal, M.F., 2002. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. J Neurosci. 22, 1592-9.

Ferrante, R.J., Kubilus, J.K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N.W., Ratan, R.R., Luthi-Carter, R., Hersch, S.M., 2003. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J Neurosci. 23, 9418-27.

Ferrante, R.J., Ryu, H., Kubilus, J.K., D'Mello, S., Sugars, K.L., Lee, J., Lu, P., Smith, K., Browne, S., Beal, M.F., Kristal, B.S., Stavrovskaya, I.G., Hewett, S., Rubinsztein, D.C., Langley, B., Ratan, R.R., 2004. Chemotherapy for the brain: the antitumor antibiotic mithramycin prolongs survival in a mouse model of Huntington's disease. J Neurosci. 24, 10335-42.

Folstein, S.E., Leigh, R.J., Parhad, I.M., Folstein, M.F., 1986. The diagnosis of Huntington's disease. Neurology. 36, 1279-83.

Folstein, S.E., 1991. The psychopathology of Huntington's disease. Research publications - Association for Research in Nervous and Mental Disease. 69, 181-91.

Frydman, J., 2001. Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu Rev Biochem. 70, 603-47.

Furtado, J.C., Mazurek, M.F., 1996. Behavioral characterization of quinolinate-induced lesions of the medial striatum: relevance for Huntington's disease. Experimental Neurology. 138, 158-68.

Futter, M., Diekmann, H., Schoenmakers, E., Sadiq, O., Chatterjee, K., Rubinsztein, D., 2009. Wild-type but not mutant huntingtin modulates the transcriptional activity of liver X receptors. J Med Genet.

Gauthier, L.R., Charrin, B.C., Borrell-Pagès, M., Dompierre, J.P., Rangone, H., Cordelières, F.P., De Mey, J., Macdonald, M.E., Lessmann, V., Humbert, S., Saudou, F., 2004. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell. 118, 127-38.

Gil, J.M., Rego, A.C., 2009. The R6 lines of transgenic mice: a model for screening new therapies for Huntington's disease. Brain research reviews. 59, 410-31.

Goldman-Rakic, P.S., 1982. Cytoarchitectonic heterogeneity of the primate neostriatum: subdivision into Island and Matrix cellular compartments. J. Comp. Neurol. 205, 398-413.

Goodman, A.O.G., Murgatroyd, P.R., Medina-Gomez, G., Wood, N.I., Finer, N., Vidal-Puig, A.J., Morton, A.J., Barker, R.A., 2008. The metabolic profile of early Huntington's disease--a combined human and transgenic mouse study. Experimental Neurology. 210, 691-8.

Gould, E., Cameron, H.A., McEwen, B.S., 1994. Blockade of NMDA receptors increases cell death and birth in the developing rat dentate gyrus. J. Comp. Neurol. 340, 551-65.

Grafton, S.T., Mazziotta, J.C., Pahl, J.J., St George-Hyslop, P., Haines, J.L., Gusella, J., Hoffman, J.M., Baxter, L.R., Phelps, M.E., 1990. A comparison of neurological, metabolic, structural, and genetic evaluations in persons at risk for Huntington's disease. Ann Neurol. 28, 614-21.

Graveland, G., Williams, R., DiFiglia, M., 1985. Evidence for Degenerative and Regenerative Changes in Neostriatal Spiny Neurons in Huntington's Disease. Science. 227, 770-773.

Gray, M., Shirasaki, D.I., Cepeda, C., André, V.M., Wilburn, B., Lu, X.-H., Tao, J., Yamazaki, I., Li, S.-H., Sun, Y.E., Li, X.-J., Levine, M.S., Yang, X.W., 2008. Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci. 28, 6182-95.

Gray, M.W., Herzberg, L., Lerman, J.A., Turnbull, M.J., Victoratos, G., 1975. Letter: Amantadine in chorea. Lancet. 2, 132-3.

Group, H.S., 2001. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. Neurology. 57, 397-404.

Group, H.S., 2003. Dosage effects of riluzole in Huntington's disease: a multicenter placebo-controlled study. Neurology. 61, 1551-6.

Group, T.H.s.D.C.R., 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, 971-83.

Gu, M., Gash, M.T., Mann, V.M., Javoy-Agid, F., Cooper, J.M., Schapira, A.H., 1996. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol. 39, 385-9.

Guidetti, P., Luthi-Carter, R.E., Augood, S.J., Schwarcz, R., 2004. Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. Neurobiology of Disease. 17, 455-61.

Guidetti, P., Bates, G.P., Graham, R.K., Hayden, M.R., Leavitt, B.R., Macdonald, M.E., Slow, E.J., Wheeler, V.C., Woodman, B., Schwarcz, R., 2006. Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice. Neurobiology of Disease. 23, 190-7.

Gunawardena, S., Her, L.-S., Brusch, R.G., Laymon, R.A., Niesman, I.R., Gordesky-Gold, B., Sintasath, L., Bonini, N.M., Goldstein, L.S.B., 2003. Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. Neuron. 40, 25-40.

Gutekunst, C.A., Li, S.H., Yi, H., Ferrante, R.J., Li, X.J., Hersch, S.M., 1998. The cellular and subcellular localization of huntingtin-associated protein 1 (HAP1): comparison with huntingtin in rat and human. J Neurosci. 18, 7674-86.

Gutekunst, C.A., Li, S.H., Yi, H., Mulroy, J.S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R.J., Hersch, S.M., Li, X.J., 1999. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci. 19, 2522-34.

Guyot, M.C., Palfi, S., Stutzmann, J.M., Mazière, M., Hantraye, P., Brouillet, E., 1997. Riluzole protects from motor deficits and striatal degeneration produced by systemic 3-nitropropionic acid intoxication in rats. Neuroscience. 81, 141-9.

Hahn-Barma, V., Deweer, B., Dürr, A., Dodé, C., Feingold, J., Pillon, B., Agid, Y., Brice, A., Dubois, B., 1998. Are cognitive changes the first symptoms of Huntington's disease? A study of gene carriers. J Neurol Neurosurg Psychiatr. 64, 172-7.

Hardingham, G.E., Fukunaga, Y., Bading, H., 2002. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat. Neurosci. 5, 405-14.

Hardingham, G.E., Bading, H., 2003. The Yin and Yang of NMDA receptor signalling. Trends Neurosci. 26, 81-9.

Hartl, F.U., Hayer-Hartl, M., 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science. 295, 1852-8.

Hayden, M.R., Martin, W.R., Stoessl, A.J., Clark, C., Hollenberg, S., Adam, M.J., Ammann, W., Harrop, R., Rogers, J., Ruth, T., 1986. Positron emission tomography in the early diagnosis of Huntington's disease. Neurology. 36, 888-94.

Heathfield, K.W., 1967. Huntington's chorea. Investigation into the prevalence of this disease in the area covered by the North East Metropolitan Regional Hospital Board. Brain. 90, 203-32.

Heckmann, J.M., Legg, P., Sklar, D., Fine, J., Bryer, A., Kies, B., 2004. IV amantadine improves chorea in Huntington's disease: an acute randomized, controlled study. Neurology. 63, 597-8; author reply 597-8.

Hedreen, J.C., Folstein, S.E., 1995. Early loss of neostriatal striosome neurons in Huntington's disease. J Neuropathol Exp Neurol. 54, 105-20.

Heng, M.Y., Tallaksen-Greene, S.J., Detloff, P.J., Albin, R.L., 2007. Longitudinal Evaluation of the Hdh(CAG)150 Knock-In Murine Model of Huntington's Disease. J Neurosci. 27, 8989-8998.

Henshaw, R., Jenkins, B.G., Schulz, J.B., Ferrante, R.J., Kowall, N.W., Rosen, B.R., Beal, M.F., 1994. Malonate produces striatal lesions by indirect NMDA receptor activation. Brain Res. 647, 161-6.

Hetman, M., Kharebava, G., 2006. Survival signaling pathways activated by NMDA receptors. Current topics in medicinal chemistry. 6, 787-99.

Hicks, S.L., Robert, M.P.A., Golding, C.V.P., Tabrizi, S.J., Kennard, C., 2008. Oculomotor deficits indicate the progression of Huntington's disease. Prog Brain Res. 171, 555-8.

Ho, A.K., Sahakian, B.J., Brown, R.G., Barker, R.A., Hodges, J.R., Ané, M.-N., Snowden, J., Thompson, J., Esmonde, T., Gentry, R., Moore, J.W., Bodner, T., Consortium, N.-H., 2003. Profile of cognitive progression in early Huntington's disease. Neurology. 61, 1702-6.

Ho, L.W., Brown, R., Maxwell, M., Wyttenbach, A., Rubinsztein, D.C., 2001. Wild type Huntingtin reduces the cellular toxicity of mutant Huntingtin in mammalian cell models of Huntington's disease. J Med Genet. 38, 450-2.

Hockly, E., Richon, V.M., Woodman, B., Smith, D.L., Zhou, X., Rosa, E., Sathasivam, K., Ghazi-Noori, S., Mahal, A., Lowden, P.A.S., Steffan, J.S., Marsh, J.L., Thompson, L.M., Lewis, C.M., Marks, P.A., Bates, G.P., 2003. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci USA. 100, 2041-6.

Hockly, E., Tse, J., Barker, A.L., Moolman, D.L., Beunard, J.-L., Revington, A.P., Holt, K., Sunshine, S., Moffitt, H., Sathasivam, K., Woodman, B., Wanker, E.E., Lowden, P.A.S., Bates, G.P., 2006. Evaluation of the benzothiazole aggregation inhibitors riluzole and PGL-135 as therapeutics for Huntington's disease. Neurobiology of Disease. 21, 228-36.

Hodgson, J.G., Agopyan, N., Gutekunst, C.A., Leavitt, B.R., LePiane, F., Singaraja, R., Smith, D.J., Bissada, N., McCutcheon, K., Nasir, J., Jamot, L., Li, X.J., Stevens, M.E., Rosemond, E., Roder, J.C., Phillips, A.G., Rubin, E.M., Hersch, S.M., Hayden, M.R., 1999. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron. 23, 181-92.

Hoffner, G., Kahlem, P., Djian, P., 2002. Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with beta-tubulin: relevance to Huntington's disease. J Cell Sci. 115, 941-8.

Huang, B., Schiefer, J., Sass, C., Landwehrmeyer, G.B., Kosinski, C.M., Kochanek, S., 2007. High-capacity adenoviral vector-mediated reduction of huntingtin aggregate load in vitro and in vivo. Hum Gene Ther. 18, 303-11.

Igarashi, S., Morita, H., Bennett, K.M., Tanaka, Y., Engelender, S., Peters, M.F., Cooper, J.K., Wood, J.D., Sawa, A., Ross, C.A., 2003. Inducible PC12 cell model of Huntington's disease shows toxicity and decreased histone acetylation. Neuroreport. 14, 565-8.

Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T.I., Stefovska, V., Turski, L., Olney, J.W., 1999. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 283, 70-4.

Jefferson, T.O., Demicheli, V., Deeks, J.J., Rivetti, D., 2001. Amantadine and rimantadine for preventing and treating influenza A in adults. Cochrane database of systematic reviews (Online). CD001169.

Jenkins, B.G., Koroshetz, W.J., Beal, M.F., Rosen, B.R., 1993. Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy. Neurology. 43, 2689-95.

Kagel, M.C., Leopold, N.A., 1992. Dysphagia in Huntington's disease: a 16-year retrospective. Dysphagia. 7, 106-14.

Kalchman, M.A., Koide, H.B., McCutcheon, K., Graham, R.K., Nichol, K., Nishiyama, K., Kazemi-Esfarjani, P., Lynn, F.C., Wellington, C., Metzler, M., Goldberg, Y.P., Kanazawa, I., Gietz, R.D., Hayden, M.R., 1997. HIP1, a human homologue of S. cerevisiae Sla2p, interacts with membrane-associated huntingtin in the brain. Nat Genet. 16, 44-53.

Kazantsev, A.G., Hersch, S.M., 2007. Drug targeting of dysregulated transcription in Huntington's disease. Prog Neurobiol. 83, 249-59.

Kingma, E.M., van Duijn, E., Timman, R., van der Mast, R.C., Roos, R.A.C., 2008. Behavioural problems in Huntington's disease using the Problem Behaviours Assessment. General hospital psychiatry. 30, 155-61.

Kirkwood, S.C., Su, J.L., Conneally, P., Foroud, T., 2001. Progression of symptoms in the early and middle stages of Huntington disease. Arch Neurol. 58, 273-8.

Kitamura, A., Kubota, H., Pack, C.-G., Matsumoto, G., Hirayama, S., Takahashi, Y., Kimura, H., Kinjo, M., Morimoto, R.I., Nagata, K., 2006. Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. Nat Cell Biol. 8, 1163-70.

Klivenyi, P., Ferrante, R.J., Gardian, G., Browne, S., Chabrier, P.-E., Beal, M.F., 2003. Increased survival and neuroprotective effects of BN82451 in a transgenic mouse model of Huntington's disease. J Neurochem. 86, 267-72.

Koroshetz, W.J., Jenkins, B.G., Rosen, B.R., Beal, M.F., 1997. Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. Ann Neurol. 41, 160-5.

Kowall, N.W., Ferrante, R.J., Beal, M.F., Richardson, E.P., Sofroniew, M.V., Cuello, A.C., Martin, J.B., 1987. Neuropeptide Y, somatostatin, and reduced nicotinamide adenine dinucleotide phosphate diaphorase in the human striatum: a combined immunocytochemical and enzyme histochemical study. Neuroscience. 20, 817-28.

Kremer, B., Goldberg, P., Andrew, S.E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., Almqvist, E., Bird, T.D., Hayden, M.R., 1994. A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. N Engl J Med. 330, 1401-6.

Kremer, B., Clark, C.M., Almqvist, E.W., Raymond, L.A., Graf, P., Jacova, C., Mezei, M., Hardy, M.A., Snow, B., Martin, W., Hayden, M.R., 1999. Influence of lamotrigine on progression of early Huntington disease: a randomized clinical trial. Neurology. 53, 1000-11.

Kremer, H.P., Roos, R.A., Dingjan, G., Marani, E., Bots, G.T., 1990. Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease. J Neuropathol Exp Neurol. 49, 371-82.

Kremer, H.P., Roos, R.A., Dingjan, G.M., Bots, G.T., Bruyn, G.W., Hofman, M.A., 1991. The hypothalamic lateral tuberal nucleus and the characteristics of neuronal loss in Huntington's disease. Neuroscience Letters. 132, 101-4.

Kuhl, D.E., Phelps, M.E., Markham, C.H., Metter, E.J., Riege, W.H., Winter, J., 1982. Cerebral metabolism and atrophy in Huntington's disease determined by 18FDG and computed tomographic scan. Ann Neurol. 12, 425-34.

Kuwert, T., Lange, H.W., Boecker, H., Titz, H., Herzog, H., Aulich, A., Wang, B.C., Nayak, U., Feinendegen, L.E., 1993. Striatal glucose consumption in chorea-free subjects at risk of Huntington's disease. J Neurol. 241, 31-6.

Lafon-Cazal, M., Perez, V., Bockaert, J., Marin, P., 2002. Akt mediates the anti-apoptotic effect of NMDA but not that induced by potassium depolarization in cultured cerebellar granule cells. Eur J Neurosci. 16, 575-83.

Laforet, G.A., Sapp, E., Chase, K., McIntyre, C., Boyce, F.M., Campbell, M., Cadigan, B.A., Warzecki, L., Tagle, D.A., Reddy, P.H., Cepeda, C., Calvert, C.R., Jokel, E.S., Klapstein, G.J., Ariano, M.A., Levine, M.S., 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, 9112-23.

Landwehrmeyer, G.B., Standaert, D.G., Testa, C.M., Penney, J.B., Young, A.B., 1995. NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. J Neurosci. 15, 5297-307.

Landwehrmeyer, G.B., Dubois, B., de Yébenes, J.G., Kremer, B., Gaus, W., Kraus, P.H., Przuntek, H., Dib, M., Doble, A., Fischer, W., Ludolph, A.C., Group, E.H.s.D.I.S., 2007. Riluzole in Huntington's disease: a 3-year, randomized controlled study. Ann Neurol. 62, 262-72.

Langbehn, D., Hayden, M., Paulsen, J., Group, a.t.P.H.I.o.t.H.S., 2009. 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.

Langbehn, D.R., Brinkman, R.R., Falush, D., Paulsen, J.S., Hayden, M.R., Group, I.H.s.D.C., 2004. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clinical Genetics. 65, 267-77.

Lange, H., Thörner, G., Hopf, A., Schröder, K.F., 1976. Morphometric studies of the neuropathological changes in choreatic diseases. J Neurol Sci. 28, 401-25.

Lawrence, A.D., Hodges, J.R., Rosser, A.E., Kershaw, A., Ffrench-Constant, C., Rubinsztein, D.C., Robbins, T.W., Sahakian, B.J., 1998a. Evidence for specific cognitive deficits in preclinical Huntington's disease. Brain. 121 (Pt 7), 1329-41.

Lawrence, A.D., Weeks, R.A., Brooks, D.J., Andrews, T.C., Watkins, L.H., Harding, A.E., Robbins, T.W., Sahakian, B.J., 1998b. The relationship between striatal dopamine receptor binding and cognitive performance in Huntington's disease. Brain. 121 (Pt 7), 1343-55.

Leavitt, B.R., Guttman, J.A., Hodgson, J.G., Kimel, G.H., Singaraja, R., Vogl, A.W., Hayden, M.R., 2001. Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo. Am J Hum Genet. 68, 313-24.

Leavitt, B.R., Van Raamsdonk, J.M., Shehadeh, J., Fernandes, H., Murphy, Z., Graham, R.K., Wellington, C.L., Raymond, L.A., Hayden, M.R., 2006. Wild-type huntingtin protects neurons from excitotoxicity. J Neurochem. 96, 1121-9.

Lee, W.-C.M., Yoshihara, M., Littleton, J.T., 2004. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. Proc Natl Acad Sci USA. 101, 3224-9.

Lerch, J.P., Carroll, J.B., Spring, S., Bertram, L.N., Schwab, C., Hayden, M.R., Henkelman, R.M., 2008. Automated deformation analysis in the YAC128 Huntington disease mouse model. Neuroimage. 39, 32-9.

Li, S.H., Gutekunst, C.A., Hersch, S.M., Li, X.J., 1998. Interaction of huntingtin-associated protein with dynactin P150Glued. J Neurosci. 18, 1261-9.

Li, X.J., Li, S.H., Sharp, A.H., Nucifora, F.C., Schilling, G., Lanahan, A., Worley, P., Snyder, S.H., Ross, C.A., 1995. A huntingtin-associated protein enriched in brain with implications for pathology. Nature. 378, 398-402.

Li, Z., Karlovich, C.A., Fish, M.P., Scott, M.P., Myers, R.M., 1999. A putative Drosophila homolog of the Huntington's disease gene. Hum Mol Genet. 8, 1807-15.

Lin, B., Nasir, J., MacDonald, H., Hutchinson, G., Graham, R.K., Rommens, J.M., Hayden, M.R., 1994. Sequence of the murine Huntington disease gene: evidence for conservation, alternate splicing and polymorphism in a triplet (CCG) repeat [corrected]. Hum Mol Genet. 3, 85-92.

Lin, C.H., Tallaksen-Greene, S., Chien, W.M., Cearley, J.A., Jackson, W.S., Crouse, A.B., Ren, S., Li, X.J., Albin, R.L., Detloff, P.J., 2001. Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet. 10, 137-44.

Liot, G., Bossy, B., Lubitz, S., Kushnareva, Y., Sejbuk, N., Bossy-Wetzel, E., 2009. Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway. Cell Death Differ. 16, 899-909.

Lucetti, C., Gambaccini, G., Bernardini, S., Dell'Agnello, G., Petrozzi, L., Rossi, G., Bonuccelli, U., 2002. Amantadine in Huntington's disease: open-label video-blinded study. Neurol Sci. 23 Suppl 2, S83-4.

Lucetti, C., Del Dotto, P., Gambaccini, G., Dell' Agnello, G., Bernardini, S., Rossi, G., Murri, L., Bonuccelli, U., 2003. IV amantadine improves chorea in Huntington's disease: an acute randomized, controlled study. Neurology. 60, 1995-7.

Luthi-Carter, R., Strand, A., Peters, N.L., Solano, S.M., Hollingsworth, Z.R., Menon, A.S., Frey, A.S., Spektor, B.S., Penney, E.B., Schilling, G., Ross, C.A., Borchelt, D.R., Tapscott, S.J., Young, A.B., Cha, J.H., Olson, J.M., 2000. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. Hum Mol Genet. 9, 1259-71.

Luthi-Carter, R., Hanson, S.A., Strand, A.D., Bergstrom, D.A., Chun, W., Peters, N.L., Woods, A.M., Chan, E.Y., Kooperberg, C., Krainc, D., Young, A.B., Tapscott, S.J., Olson, J.M.,

2002a. Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum Mol Genet. 11, 1911-26.

Luthi-Carter, R., Strand, A.D., Hanson, S.A., Kooperberg, C., Schilling, G., La Spada, A.R., Merry, D.E., Young, A.B., Ross, C.A., Borchelt, D.R., Olson, J.M., 2002b. Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington's disease mouse models reveal context-independent effects. Hum Mol Genet. 11, 1927-37.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W., Bates, G.P., 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, V.M., Cooper, J.M., Javoy-Agid, F., Agid, Y., Jenner, P., Schapira, A.H., 1990. Mitochondrial function and parental sex effect in Huntington's disease. Lancet. 336, 749.

Mao, Z., Choo, Y.S., Lesort, M., 2006. Cystamine and cysteamine prevent 3-NP-induced mitochondrial depolarization of Huntington's disease knock-in striatal cells. Eur J Neurosci. 23, 1701-10.

Markianos, M., Panas, M., Kalfakis, N., Vassilopoulos, D., 2005. Plasma testosterone in male patients with Huntington's disease: relations to severity of illness and dementia. Ann Neurol. 57, 520-5.

Mary, V., Wahl, F., Stutzmann, J.M., 1995. Effect of riluzole on quinolinate-induced neuronal damage in rats: comparison with blockers of glutamatergic neurotransmission. Neuroscience Letters. 201, 92-6.

Mastroberardino, P.G., Iannicola, C., Nardacci, R., Bernassola, F., De Laurenzi, V., Melino, G., Moreno, S., Pavone, F., Oliverio, S., Fesus, L., Piacentini, M., 2002. 'Tissue' transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. Cell Death Differ. 9, 873-80.

Mazziotta, J.C., Phelps, M.E., Pahl, J.J., Huang, S.C., Baxter, L.R., Riege, W.H., Hoffman, J.M., Kuhl, D.E., Lanto, A.B., Wapenski, J.A., 1987. Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. N Engl J Med. 316, 357-62.

McGeer, E.G., McGeer, P.L., 1976. Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature. 263, 517-9.

McNeil, S.M., Novelletto, A., Srinidhi, J., Barnes, G., Kornbluth, I., Altherr, M.R., Wasmuth, J.J., Gusella, J.F., MacDonald, M.E., Myers, R.H., 1997. Reduced penetrance of the Huntington's disease mutation. Hum Mol Genet. 6, 775-9.

Menalled, L.B., Sison, J.D., Wu, Y., Olivieri, M., Li, X.-J., Li, H., Zeitlin, S., Chesselet, M.-F., 2002. Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. J Neurosci. 22, 8266-76.

Menalled, L.B., Sison, J.D., Dragatsis, I., Zeitlin, S., Chesselet, M.-F., 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.

Metzler, M., Chen, N., Helgason, C.D., Graham, R.K., Nichol, K., McCutcheon, K., Nasir, J., Humphries, R.K., Raymond, L.A., Hayden, M.R., 1999. Life without huntingtin: normal differentiation into functional neurons. J Neurochem. 72, 1009-18.

Metzler, M., Helgason, C.D., Dragatsis, I., Zhang, T., Gan, L., Pineault, N., Zeitlin, S.O., Humphries, R.K., Hayden, M.R., 2000. Huntingtin is required for normal hematopoiesis. Hum Mol Genet. 9, 387-94.

Metzler, M., Legendre-Guillemin, V., Gan, L., Chopra, V., Kwok, A., McPherson, P.S., Hayden, M.R., 2001. HIP1 functions in clathrin-mediated endocytosis through binding to clathrin and adaptor protein 2. J Biol Chem. 276, 39271-6.

Milnerwood, A.J., Gladding, C.M., Pouladi, M.A., Kaufman, A.M., Hines, R.M., Boyd, J.D., Ko, R.W., Vasuta, O.C., Graham, R.K., Hayden, M.R., Murphy, T.H., Raymond, L.A., 2010. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron. 65, 178-90.

Monti, B., Contestabile, A., 2000. Blockade of the NMDA receptor increases developmental apoptotic elimination of granule neurons and activates caspases in the rat cerebellum. Eur J Neurosci. 12, 3117-23.

Morfini, G., You, Y., Pollema, S., Kaminska, A., Liu, K., Yoshioka, K., Björkblom, B., Coffey, E., Bagnato, C., Han, D., Huang, C., Banker, G., Pigino, G., Brady, S., 2009. Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin. Nat. Neurosci.

Morton, A.J., Lagan, M.A., Skepper, J.N., Dunnett, S.B., 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.

Muchowski, P.J., 2002. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron. 35, 9-12.

Murman, D.L., Giordani, B., Mellow, A.M., Johanns, J.R., Little, R.J., Hariharan, M., Foster, N.L., 1997. Cognitive, behavioral, and motor effects of the NMDA antagonist ketamine in Huntington's disease. Neurology. 49, 153-61.

Myers, R.H., Sax, D.S., Koroshetz, W.J., Mastromauro, C., Cupples, L.A., Kiely, D.K., Pettengill, F.K., Bird, E.D., 1991. Factors associated with slow progression in Huntington's disease. Arch Neurol. 48, 800-4.

Nance, M.A., Mathias-Hagen, V., Breningstall, G., Wick, M.J., McGlennen, R.C., 1999. Analysis of a very large trinucleotide repeat in a patient with juvenile Huntington's disease. Neurology. 52, 392-4.

Nasir, J., Floresco, S.B., O'Kusky, J.R., Diewert, V.M., Richman, J.M., Zeisler, J., Borowski, A., Marth, J.D., Phillips, A.G., Hayden, M.R., 1995. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell. 81, 811-23.

Nollen, E.A.A., Garcia, S.M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R.I., Plasterk, R.H.A., 2004. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. Proc Natl Acad Sci USA. 101, 6403-8.

Nørremølle, A., Riess, O., Epplen, J.T., Fenger, K., Hasholt, L., Sørensen, S.A., 1993. Trinucleotide repeat elongation in the Huntingtin gene in Huntington disease patients from 71 Danish families. Hum Mol Genet. 2, 1475-6.

Novelli, A., Reilly, J.A., Lysko, P.G., Henneberry, R.C., 1988. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 451, 205-12.

Obrietan, K., Hoyt, K.R., 2004. CRE-mediated transcription is increased in Huntington's disease transgenic mice. J Neurosci. 24, 791-6.

Okuda, S., Nishiyama, N., Saito, H., Katsuki, H., 1996. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. Proc Natl Acad Sci USA. 93, 12553-8.

Ona, V.O., Li, M., Vonsattel, J.P., Andrews, L.J., Khan, S.Q., Chung, W.M., Frey, A.S., Menon, A.S., Li, X.J., Stieg, P.E., Yuan, J., Penney, J.B., Young, A.B., Cha, J.H., Friedlander, R.M., 1999. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. Nature. 399, 263-7.

Ondo, W.G., Mejia, N.I., Hunter, C.B., 2007. A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. Parkinsonism Relat Disord. 13, 453-4.

Ordway, J.M., Tallaksen-Greene, S., Gutekunst, C.A., Bernstein, E.M., Cearley, J.A., Wiener, H.W., Dure, L.S., Lindsey, R., Hersch, S.M., Jope, R.S., Albin, R.L., Detloff, P.J., 1997. Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. Cell. 91, 753-63.

Palfi, S., Riche, D., Brouillet, E., Guyot, M.C., Mary, V., Wahl, F., Peschanski, M., Stutzmann, J.M., Hantraye, P., 1997. Riluzole reduces incidence of abnormal movements but not striatal cell death in a primate model of progressive striatal degeneration. Experimental Neurology. 146, 135-41.

Pallos, J., Bodai, L., Lukacsovich, T., Purcell, J.M., Steffan, J.S., Thompson, L.M., Marsh, J.L., 2008. Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a Drosophila model of Huntington's disease. Hum Mol Genet. 17, 3767-75.

Panov, A.V., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., Burke, J.R., Strittmatter, W.J., Greenamyre, J.T., 2002. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat. Neurosci. 5, 731-6.

Papadia, S., Stevenson, P., Hardingham, N.R., Bading, H., Hardingham, G.E., 2005. Nuclear Ca2+ and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. J Neurosci. 25, 4279-87.

Papadia, S., Soriano, F.X., Léveillé, F., Martel, M.-A., Dakin, K.A., Hansen, H.H., Kaindl, A., Sifringer, M., Fowler, J., Stefovska, V., McKenzie, G., Craigon, M., Corriveau, R., Ghazal, P., Horsburgh, K., Yankner, B.A., Wyllie, D.J.A., Ikonomidou, C., Hardingham, G.E., 2008. Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. Nat. Neurosci. 11, 476-87.

Paulsen, J.S., Ready, R.E., Hamilton, J.M., Mega, M.S., Cummings, J.L., 2001. Neuropsychiatric aspects of Huntington's disease. J Neurol Neurosurg Psychiatr. 71, 310-4.

Pflanz, S., Besson, J.A., Ebmeier, K.P., Simpson, S., 1991. The clinical manifestation of mental disorder in Huntington's disease: a retrospective case record study of disease progression. Acta psychiatrica Scandinavica. 83, 53-60.

Pillon, B., Dubois, B., Agid, Y., 1991. Severity and specificity of cognitive impairment in Alzheimer's, Huntington's, and Parkinson's diseases and progressive supranuclear palsy. Annals of the New York Academy of Sciences. 640, 224-7.

Podoll, K., Caspary, P., Lange, H.W., Noth, J., 1988. Language functions in Huntington's disease. Brain. 111 (Pt 6), 1475-503.

Pohl, D., Bittigau, P., Ishimaru, M.J., Stadthaus, D., Hübner, C., Olney, J.W., Turski, L., Ikonomidou, C., 1999. N-Methyl-D-aspartate antagonists and apoptotic cell death triggered by head trauma in developing rat brain. Proc Natl Acad Sci USA. 96, 2508-13.

Popoli, P., Pèzzola, A., Domenici, M.R., Sagratella, S., Diana, G., Caporali, M.G., Bronzetti, E., Vega, J., Scotti de Carolis, A., 1994. Behavioral and electrophysiological correlates of the quinolinic acid rat model of Huntington's disease in rats. Brain Res Bull. 35, 329-35.

R. Hayden, M., 1981. Huntington's Chorea.

Reddy, P.H., Charles, V., Williams, M., Miller, G., Whetsell, W.O., Tagle, D.A., 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, 1035-45.

Reiner, A., Del Mar, N., Meade, C.A., Yang, H., Dragatsis, I., Zeitlin, S., Goldowitz, D., 2001. Neurons lacking huntingtin differentially colonize brain and survive in chimeric mice. J Neurosci. 21, 7608-19.

Reiner, A., Del Mar, N., Deng, Y.-P., Meade, C.A., 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, 603-29.

Rigamonti, D., Bauer, J.H., De-Fraja, C., Conti, L., Sipione, S., Sciorati, C., Clementi, E., Hackam, A., Hayden, M.R., Li, Y., Cooper, J.K., Ross, C.A., Govoni, S., Vincenz, C.,

Cattaneo, E., 2000. Wild-type huntingtin protects from apoptosis upstream of caspase-3. J Neurosci. 20, 3705-13.

Rigamonti, D., Sipione, S., Goffredo, D., Zuccato, C., Fossale, E., Cattaneo, E., 2001. Huntingtin's neuroprotective activity occurs via inhibition of procaspase-9 processing. J Biol Chem. 276, 14545-8.

Robbins, A.O., Ho, A.K., Barker, R.A., 2006. Weight changes in Huntington's disease. Eur J Neurol. 13, e7.

Rodda, R.A., 1981. Cerebellar atrophy in Huntington's disease. J Neurol Sci. 50, 147-57.

Roos, R.A., Bots, G.T., Hermans, J., 1986. Quantitative analysis of morphological features in Huntington's disease. Acta Neurol Scand. 73, 131-5.

Rosas, H.D., Koroshetz, W.J., Jenkins, B.G., Chen, Y.I., Hayden, D.L., Beal, M.F., Cudkowicz, M.E., 1999. Riluzole therapy in Huntington's disease (HD). Mov Disord. 14, 326-30.

Roze, E., Saudou, F., Caboche, J., 2008. Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. Curr Opin Neurol. 21, 497-503.

Ruan, Q., Lesort, M., Macdonald, M.E., Johnson, G.V.W., 2004. Striatal cells from mutant huntingtin knock-in mice are selectively vulnerable to mitochondrial complex II inhibitor-induced cell death through a non-apoptotic pathway. Hum Mol Genet. 13, 669-81.

Sadri-Vakili, G., Bouzou, B., Benn, C.L., Kim, M.-O., Chawla, P., Overland, R.P., Glajch, K.E., Xia, E., Qiu, Z., Hersch, S.M., Clark, T.W., Yohrling, G.J., Cha, J.-H.J., 2007. Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. Hum Mol Genet. 16, 1293-306.

Sahlender, D.A., Roberts, R.C., Arden, S.D., Spudich, G., Taylor, M.J., Luzio, J.P., Kendrick-Jones, J., Buss, F., 2005. Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. The Journal of Cell Biology. 169, 285-95.

Sanberg, P.R., Fibiger, H.C., Mark, R.F., 1981. Body weight and dietary factors in Huntington's disease patients compared with matched controls. Med J Aust. 1, 407-9.

Sánchez, I., Mahlke, C., Yuan, J., 2003. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. Nature. 421, 373-9.

Sawa, A., Wiegand, G.W., Cooper, J., Margolis, R.L., Sharp, A.H., Lawler, J.F., Greenamyre, J.T., Snyder, S.H., Ross, C.A., 1999. Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. Nat Med. 5, 1194-8.

Scherzinger, E., Lurz, R., Turmaine, M., Mangiarini, L., Hollenbach, B., Hasenbank, R., Bates, G.P., Davies, S.W., Lehrach, H., Wanker, E.E., 1997. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell. 90, 549-58.

Schilling, G., Becher, M.W., Sharp, A.H., Jinnah, H.A., Duan, K., Kotzuk, J.A., Slunt, H.H., Ratovitski, T., Cooper, J.K., Jenkins, N.A., Copeland, N.G., Price, D.L., Ross, C.A.,

Borchelt, D.R., 1999. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet. 8, 397-407.

Schilling, G., Coonfield, M.L., Ross, C.A., Borchelt, D.R., 2001. Coenzyme Q10 and remacemide hydrochloride ameliorate motor deficits in a Huntington's disease transgenic mouse model. Neuroscience Letters. 315, 149-53.

Schmitt, I., Bächner, D., Megow, D., Henklein, P., Hameister, H., Epplen, J.T., 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, 1173-82.

Schulz, J.B., Dichgans, J., 1999. Molecular pathogenesis of movement disorders: are protein aggregates a common link in neuronal degeneration? Curr Opin Neurol. 12, 433-9.

Seppi, K., Mueller, J., Bodner, T., Brandauer, E., Benke, T., Weirich-Schwaiger, H., Poewe, W., Wenning, G.K., 2001. Riluzole in Huntington's disease (HD): an open label study with one year follow up. J Neurol. 248, 866-9.

Shear, D.A., Dong, J., Gundy, C.D., Haik-Creguer, K.L., Dunbar, G.L., 1998. Comparison of intrastriatal injections of quinolinic acid and 3-nitropropionic acid for use in animal models of Huntington's disease. Prog Neuropsychopharmacol Biol Psychiatry. 22, 1217-40.

Shehadeh, J., Fernandes, H.B., Zeron Mullins, M.M., Graham, R.K., Leavitt, B.R., Hayden, M.R., Raymond, L.A., 2006. Striatal neuronal apoptosis is preferentially enhanced by NMDA receptor activation in YAC transgenic mouse model of Huntington disease. Neurobiology of Disease. 21, 392-403.

Shelbourne, P.F., Killeen, N., Hevner, R.F., Johnston, H.M., Tecott, L., Lewandoski, M., Ennis, M., Ramirez, L., Li, Z., Iannicola, C., Littman, D.R., Myers, R.M., 1999. A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. Hum Mol Genet. 8, 763-74.

Sipione, S., Rigamonti, D., Valenza, M., Zuccato, C., Conti, L., Pritchard, J., Kooperberg, C., Olson, J.M., Cattaneo, E., 2002. Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Hum Mol Genet. 11, 1953-65.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12, 1555-67.

Slow, E.J., Graham, R.K., Osmand, A.P., Devon, R.S., Lu, G., Deng, Y., Pearson, J., Vaid, K., Bissada, N., Wetzel, R., Leavitt, B.R., Hayden, M.R., 2005. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. Proc Natl Acad Sci USA. 102, 11402-7.

Slow, E.J., Graham, R.K., Hayden, M.R., 2006. To be or not to be toxic: aggregations in Huntington and Alzheimer disease. Trends Genet. 22, 408-11.

Snell, R.G., MacMillan, J.C., Cheadle, J.P., Fenton, I., Lazarou, L.P., Davies, P., MacDonald, M.E., Gusella, J.F., Harper, P.S., Shaw, D.J., 1993. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nat Genet. 4, 393-7.

Snowden, J., Craufurd, D., Griffiths, H., Thompson, J., Neary, D., 2001. Longitudinal evaluation of cognitive disorder in Huntington's disease. Journal of the International Neuropsychological Society : JINS. 7, 33-44.

Snowden, J.S., Craufurd, D., Thompson, J., Neary, D., 2002. Psychomotor, executive, and memory function in preclinical Huntington's disease. Journal of clinical and experimental neuropsychology. 24, 133-45.

Sotrel, A., Paskevich, P.A., Kiely, D.K., Bird, E.D., Williams, R.S., Myers, R.H., 1991. Morphometric analysis of the prefrontal cortex in Huntington's disease. Neurology. 41, 1117-23.

Spokes, E.G., 1980. Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain. 103, 179-210.

Squitieri, F., Gellera, C., Cannella, M., Mariotti, C., Cislaghi, G., Rubinsztein, D.C., Almqvist, E.W., Turner, D., Bachoud-Lévi, A.-C., Simpson, S.A., Delatycki, M., Maglione, V., Hayden, M.R., Donato, S.D., 2003. Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. Brain. 126, 946-55.

Squitieri, F., Ciammola, A., Colonnese, C., Ciarmiello, A., 2008. Neuroprotective effects of riluzole in Huntington's disease. Eur J Nucl Med Mol Imaging. 35, 221-2.

Starr, A., 1967. A disorder of rapid eye movements in Huntington's chorea. Brain. 90, 545-64.

Steffan, J.S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B.L., Kazantsev, A., Schmidt, E., Zhu, Y.Z., Greenwald, M., Kurokawa, R., Housman, D.E., Jackson, G.R., Marsh, J.L., Thompson, L.M., 2001. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature. 413, 739-43.

Stoof, J.C., Booij, J., Drukarch, B., 1992a. Amantadine as N-methyl-D-aspartic acid receptor antagonist: new possibilities for therapeutic applications? Clinical neurology and neurosurgery. 94 Suppl, S4-6.

Stoof, J.C., Booij, J., Drukarch, B., Wolters, E.C., 1992b. The anti-parkinsonian drug amantadine inhibits the N-methyl-D-aspartic acid-evoked release of acetylcholine from rat neostriatum in a non-competitive way. Eur J Pharmacol. 213, 439-43.

Strehlow, A.N.T., Li, J.Z., Myers, R.M., 2007. Wild-type huntingtin participates in protein trafficking between the Golgi and the extracellular space. Hum Mol Genet. 16, 391-409.

Sugars, K.L., Rubinsztein, D.C., 2003. Transcriptional abnormalities in Huntington disease. Trends Genet. 19, 233-8.

Szebenyi, G., Morfini, G.A., Babcock, A., Gould, M., Selkoe, K., Stenoien, D.L., Young, M., Faber, P.W., Macdonald, M.E., McPhaul, M.J., Brady, S.T., 2003. Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. Neuron. 40, 41-52.

Tabrizi, S.J., Cleeter, M.W., Xuereb, J., Taanman, J.W., Cooper, J.M., Schapira, A.H., 1999. Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Ann Neurol. 45, 25-32.

Tagliavini, F., Pilleri, G., 1983. Basal nucleus of Meynert. A neuropathological study in Alzheimer's disease, simple senile dementia, Pick's disease and Huntington's chorea. J Neurol Sci. 62, 243-60.

Tam, S., Geller, R., Spiess, C., Frydman, J., 2006. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol. 8, 1155-62.

Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N.R., Doi, H., Kurosawa, M., Nekooki, M., Nukina, N., 2004. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. Nat Med. 10, 148-54.

Tang, T.-S., Slow, E., Lupu, V., Stavrovskaya, I.G., Sugimori, M., Llinás, R., Kristal, B.S., Hayden, M.R., Bezprozvanny, I., 2005. Disturbed Ca2+ signaling and apoptosis of medium spiny neurons in Huntington's disease. Proc Natl Acad Sci USA. 102, 2602-7.

Thomas, E.A., Coppola, G., Desplats, P.A., Tang, B., Soragni, E., Burnett, R., Gao, F., Fitzgerald, K.M., Borok, J.F., Herman, D., Geschwind, D.H., Gottesfeld, J.M., 2008. The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. Proc Natl Acad Sci USA. 105, 15564-9.

Trejo, A., Tarrats, R.M., Alonso, M.E., Boll, M.-C., Ochoa, A., Velásquez, L., 2004. Assessment of the nutrition status of patients with Huntington's disease. Nutrition (Burbank, Los Angeles County, Calif). 20, 192-6.

Trushina, E., Dyer, R.B., Badger, J.D., Ure, D., Eide, L., Tran, D.D., Vrieze, B.T., Legendre-Guillemin, V., McPherson, P.S., Mandavilli, B.S., Van Houten, B., Zeitlin, S., McNiven, M., Aebersold, R., Hayden, M., Parisi, J.E., Seeberg, E., Dragatsis, I., Doyle, K., Bender, A., Chacko, C., McMurray, C.T., 2004. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Mol Cell Biol. 24, 8195-209.

Tukamoto, T., Nukina, N., Ide, K., Kanazawa, I., 1997. Huntington's disease gene product, huntingtin, associates with microtubules in vitro. Brain Res Mol Brain Res. 51, 8-14.

van der Burg, J.M., Björkqvist, M., Brundin, P., 2009. Beyond the brain: widespread pathology in Huntington's disease. Lancet neurology. 8, 765-74.

Van Raamsdonk, J.M., Pearson, J., Slow, E.J., Hossain, S.M., Leavitt, B.R., Hayden, M.R., 2005. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci. 25, 4169-80.

Van Raamsdonk, J.M., Murphy, Z., Selva, D.M., Hamidizadeh, R., Pearson, J., Petersén, A., Björkqvist, M., Muir, C., Mackenzie, I.R., Hammond, G.L., Vogl, A.W., Hayden, M.R.,

Leavitt, B.R., 2007. Testicular degeneration in Huntington disease. Neurobiology of Disease. 26, 512-20.

Velier, J., Kim, M., Schwarz, C., Kim, T.W., Sapp, E., Chase, K., Aronin, N., Difiglia, M., 1998. Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways. Experimental Neurology. 152, 34-40.

Verhagen Metman, L., Morris, M.J., Farmer, C., Gillespie, M., Mosby, K., Wuu, J., Chase, T.N., 2002. Huntington's disease: a randomized, controlled trial using the NMDA-antagonist amantadine. Neurology. 59, 694-9.

Vonsattel, J.P., Myers, R.H., Stevens, T.J., Ferrante, R.J., Bird, E.D., Richardson, E.P., 1985. Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol. 44, 559-77.

Vonsattel, J.P., Difiglia, M., 1998. Huntington disease. J Neuropathol Exp Neurol. 57, 369-84.

Wang, H., Lim, P.J., Karbowski, M., Monteiro, M.J., 2009. Effects of overexpression of huntingtin proteins on mitochondrial integrity. Hum Mol Genet. 18, 737-52.

Wanker, E.E., Rovira, C., Scherzinger, E., Hasenbank, R., Wälter, S., Tait, D., Colicelli, J., Lehrach, H., 1997. HIP-I: a huntingtin interacting protein isolated by the yeast two-hybrid system. Hum Mol Genet. 6, 487-95.

Wellington, C.L., Leavitt, B.R., Hayden, M.R., 2000. Huntington disease: new insights on the role of huntingtin cleavage. J Neural Transm Suppl. 1-17.

Weydt, P., Pineda, V.V., Torrence, A.E., Libby, R.T., Satterfield, T.F., Lazarowski, E.R., Gilbert, M.L., Morton, G.J., Bammler, T.K., Strand, A.D., Cui, L., Beyer, R.P., Easley, C.N., Smith, A.C., Krainc, D., Luquet, S., Sweet, I.R., Schwartz, M.W., La Spada, A.R., 2006. Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab. 4, 349-62.

Wheeler, V.C., White, J.K., Gutekunst, C.A., Vrbanac, V., Weaver, M., Li, X.J., Li, S.H., Yi, H., Vonsattel, J.P., Gusella, J.F., Hersch, S., Auerbach, W., Joyner, A.L., MacDonald, M.E., 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, 503-13.

Whetsell, W.O., Schwarcz, R., 1989. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. Neuroscience Letters. 97, 271-5.

White, J.K., Auerbach, W., Duyao, M.P., Vonsattel, J.P., Gusella, J.F., Joyner, A.L., MacDonald, M.E., 1997. Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet. 17, 404-10.

Wiegand, M., Möller, A.A., Lauer, C.J., Stolz, S., Schreiber, W., Dose, M., Krieg, J.C., 1991a. Nocturnal sleep in Huntington's disease. J Neurol. 238, 203-8.

Wiegand, M., Möller, A.A., Schreiber, W., Lauer, C., Krieg, J.C., 1991b. Brain morphology and sleep EEG in patients with Huntington's disease. Eur Arch Psychiatry Clin Neurosci. 240, 148-52.

Witjes-Ané, M.-N.W., Vegter-van der Vlis, M., van Vugt, J.P.P., Lanser, J.B.K., Hermans, J., Zwinderman, A.H., van Ommen, G.-J.B., Roos, R.A.C., 2003. Cognitive and motor functioning in gene carriers for Huntington's disease: a baseline study. The Journal of neuropsychiatry and clinical neurosciences. 15, 7-16.

Wood, N.I., Pallier, P.N., Wanderer, J., Morton, A.J., 2007. Systemic administration of Congo red does not improve motor or cognitive function in R6/2 mice. Neurobiology of Disease. 25, 342-53.

Yamamoto, A., Lucas, J.J., Hen, R., 2000. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell. 101, 57-66.

Young, A.B., Greenamyre, J.T., Hollingsworth, Z., Albin, R., D'Amato, C., Shoulson, I., Penney, J.B., 1988. NMDA receptor losses in putamen from patients with Huntington's disease. Science. 241, 981-3.

Zeevalk, G.D., Nicklas, W.J., 1991. Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. J Pharmacol Exp Ther. 257, 870-8.

Zeitlin, S., Liu, J.P., Chapman, D.L., Papaioannou, V.E., Efstratiadis, A., 1995. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. Nat Genet. 11, 155-63.

Zeron, M.M., Chen, N., Moshaver, A., Lee, A.T., Wellington, C.L., Hayden, M.R., Raymond, L.A., 2001. Mutant huntingtin enhances excitotoxic cell death. Mol Cell Neurosci. 17, 41-53.

Zeron, M.M., Hansson, O., Chen, N., Wellington, C.L., Leavitt, B.R., Brundin, P., Hayden, M.R., Raymond, L.A., 2002. Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron. 33, 849-60.

Zeron, M.M., Fernandes, H.B., Krebs, C., Shehadeh, J., Wellington, C.L., Leavitt, B.R., Baimbridge, K.G., Hayden, M.R., Raymond, L.A., 2004. Potentiation of NMDA receptor-mediated excitotoxicity linked with intrinsic apoptotic pathway in YAC transgenic mouse model of Huntington's disease. Mol Cell Neurosci. 25, 469-79.

Zhai, W., Jeong, H., Cui, L., Krainc, D., Tjian, R., 2005. In vitro analysis of huntingtin-mediated transcriptional repression reveals multiple transcription factor targets. Cell. 123, 1241-53.

Zhang, S., Feany, M.B., Saraswati, S., Littleton, J.T., Perrimon, N., 2009. Inactivation of Drosophila Huntingtin affects long-term adult functioning and the pathogenesis of a Huntington's disease model. Dis Model Mech. 2, 247-66.

Zhang, S.-J., Steijaert, M.N., Lau, D., Schütz, G., Delucinge-Vivier, C., Descombes, P., Bading, H., 2007. Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. Neuron. 53, 549-62.

Zhang, Y., Leavitt, B.R., Van Raamsdonk, J.M., Dragatsis, I., Goldowitz, D., Macdonald, M.E., Hayden, M.R., Friedlander, R.M., 2006. Huntingtin inhibits caspase-3 activation. EMBO J. 25, 5896-906.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B.R., Goffredo, D., Conti, L., MacDonald, M.E., Friedlander, R.M., Silani, V., Hayden, M.R., Timmusk, T., Sipione, S., Cattaneo, E., 2001. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science. 293, 493-8.

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

2. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingin[1]

2.1. Introduction

Huntington disease (HD) is an inherited neurodegenerative disorder caused by an expansion of a polyglutamine repeat in the N-terminal region of huntingtin (Group, 1993). Aggregation of mutant huntingtin (mtHtt) into insoluble macro inclusions, and early selective cell death of striatal and cortical neurons are features of the disease (DiFiglia et al., 1997). Interestingly, most if not all neurodegenerative disorders, whether or not genetically inherited like HD, manifest abnormal misfolded proteins (Ciechanover and Brundin, 2003). Although it has been known for many years that excessive NMDAR activity can mimic HD (Beal et al., 1986) and that such activity might contribute to disease pathogenesis (Ferrante et al., 2002; Heng et al., 2009; Zeron et al., 2002), it remains unknown if normal synaptic activity influences inclusion formation and neuronal survival. Additionally, mechanistic details relating electrophysiological activity and molecular pathways to protein misfolding and aggregation in disorders such as HD have been lacking. Moreover, a mechanism-based treatment has not yet proven successful in HD patients.

2.2. Materials and methods

Cell culture and transfection. Primary cerebrocortical or striatal cultures, containing both neurons and glia, were made from E16 rat pups and grown on 12-mm glass coverslips, as we have described (Okamoto et al., 2002). Cells were transfected via LipofectAMINE 2000

[1] A version of this chapter has been published. Okamoto SI*, Pouladi MA*, Talantova M*, Yao D, Xia P, Ehrnhoefer DE, Zaidi R, Clemente A, Kaul M, Graham R, Zhang D, Vincent Chen HS, Tong G, Hayden MR**, Lipton SA**. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. Nat Med (2009) vol. 15 pp. 1407 – 1413 (* co-first authors; ** co-senior authors)

(Invitrogen) on the 15-20th DIV with either N-terminal fragments of Htt consisting of exon 1 (Myc-wtHtt-N63-18Q or Myc-mtHtt-N63-148Q), or with full-length Htt (wtHtt with 15Q or mtHtt with 138Q). In a number of experiments, cells were co-transfected with enhanced green fluorescence protein (EGFP) to facilitate identification of transduced cells. Labeled cells could be subsequently stained with anti-NeuN or anti-MAP-2 antibody, verifying their neuronal identity. In a series of experiments, a PGC-1α expression vector (ATCC) was also co-transfected. Transfection efficiency was 10-15% for cortical neurons, and 5-10% for striatal neurons. In experiments manipulating synaptic activity, drugs were added to cultures five hours post transfection. Extracellular Mg^{2+} was set at $\leq$ 0.4 mM to produce spontaneous NMDAR activity in the absence of excessive AMPAR stimulation, as assessed by patch-clamp recordings (Lei et al., 1992). CREB transcriptional activity was analyzed using a CRE luciferase reporter (Stratagene) and renilla luciferase internal control vector (Promega), as previously described (Okamoto et al., 2002). siSTRIKE plasmids expressing shRNAs targeting TCP1 and a non-targeted sequence were constructed according to the manufacturer's protocol (Promega). TCP1 shRNA targeting the sequences were designed using Promega's siRNA Target Designer.

Assessment of inclusion formation and neuronal cell death. For inclusion assessment, Htt-transfected cortical or striatal neurons were stained with anti-myc (for truncated N-terminal Htt constructs, Santa Cruz), or anti-huntingtin (for full-length Htt constructs, Chemicon) to visualize huntingtin expression under deconvolution microscopy. The expression level of transfected full-length Htt was sufficiently above baseline to allow us to easily distinguish transfected from endogenous Htt (Figure 2.22). Images were captured with a Zeiss Axiovert 100M microscope equipped with deconvolution software (see Deconvolution experimental procedures, below).

Inclusion formation was assessed 15-17 h after drug application. Immunocytochemistry for MAP2 (Sigma) and NeuN (Chemicon) were used to identify neurons. The total number of transfected neurons with Htt-positive inclusions and the total number of transfected neurons on each coverslip were counted. In a series of experiments combining inclusion and neuronal cell death analysis, wtHtt or mtHtt was co-transfected with EGFP, the latter to further facilitate identification of all transduced cells; in this manner, we could normalize the number of viable, dead, or inclusion-containing neurons to the total number of transfected neurons for each treatment paradigm. In the cell death experiments, wtHtt- or mtHtt-expressing neurons were incubated in media with or without 50 μM glutamate for 40 h. In another series of experiments, cultures were incubated for 20 h with TTX or NO-711. In some experiments, PGC-1α was also expressed. Cells were then stained with anti-MAP2/anti-NeuN, anti-myc antibodies, anti-PGC-1α antibody (Santa Cruz), anti-TCP1 antibody (Santa Cruz), and Hoechst dye 33342. Neuronal cell death was assessed on the basis of condensed nuclear morphology (with Hoechst dye 33342) and shrunken neurites. Neurons were scored as viable if they possessed normal nuclei and neuritic processes (Nucifora et al., 2001). In both the inclusion formation and neuronal death assessments, for each experimental condition, ≥100 neurons were counted on triplicate coverslips, and experiments were repeated at least four times; thus, $n \geq 1200$ in each case. Data are expressed as mean ± s.e.m., and statistical significance was determined using an ANOVA followed by Dunnett's post-hoc test.

Deconvolution microscopy and fluorescence intensity measurements. Digital images were acquired using a CCD camera (Cooke Sensicam) mounted on a Zeiss Axiovert 100M epifluorescence microscope (63x oil-immersion objective, 1.4 NA), equipped with SlideBook deconvolution software (Intelligent Imaging Innovations). Reconstructed images were generated

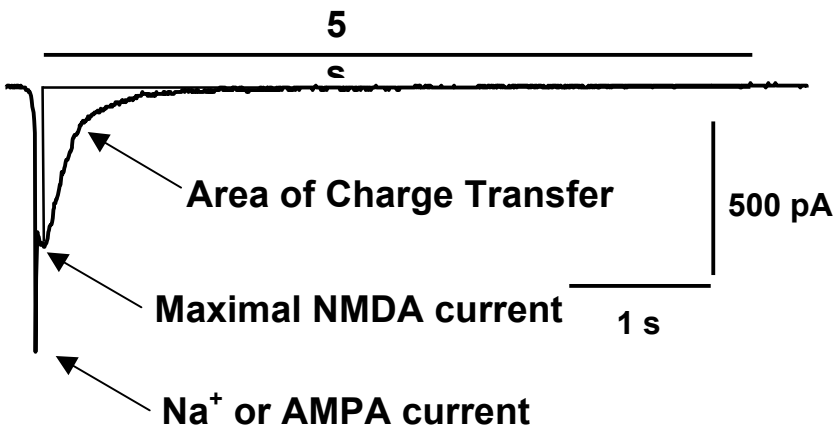
from stacks of 10-20 z-axis images of 0.3 μm thick optical sections deconvolved with a nearest neighbor algorithm. For fluorescence intensity measurements, immunostaining and image capturing were performed under the same conditions for each experiment; 20-31 cells from each experiment were randomly selected, and the average fluorescence intensity was determined by measuring the signal from the cell body using SlideBook software. Data were then normalized and evaluated by ANOVA. For mtHtt-transfected neurons, the number of inclusion bodies (inclusions) in each cell was counted, and, for each observed inclusion, the mean intensity and area were digitally measured.

Western blot analysis. Lysates of cortical or striatal cultures were prepared in phosphate buffered saline (PBS) with 1% sodium dodecyl sulfate (SDS), and then subjected to Western blot analysis and probed with anti-Myc antibody (Santa Cruz) to detect the expression of huntingtin proteins.

Electrophysiological recordings. Whole-cell recordings were performed with an Axopatch 200B amplifier (Axon Instruments) from EGFP-positive neurons 48 to 96 h after transfection. The extracellular solution contained (in mM): NaCl 135, KCl 2.5, $CaCl_2$ 2, $MgCl_2$ 0-0.01, $NaHCO_3$ 1, Na_2HPO_4 0.34, KH_2PO_4 0.44, glucose 20, Hepes 10, pH 7.4. Patch electrodes with a final tip resistance of 4-7 MΩ, were filled with a solution containing (in mM): CsCl 120, tetraethylammonium chloride (TEA-Cl) 20, Hepes 10, EGTA 2.25, $CaCl_2$ 1, $MgCl_2$ 2, and phalloidin 0.001, pH 7.4. To examine NMDAR-mediated sEPSCs, we used "charge transfer." Details of this analysis are given in the Analysis of NMDAR-mediated sEPSCs section below and in Figure 2.12. pCLAMP 8-9.2 software (Axon Instruments) was used for data acquisition and analysis. All recordings were made at room temperature with a holding potential of -60 mV. Currents were digitally sampled at 10-20 kHz and filtered at 2-5 kHz. Data are expressed as

mean ± s.e.m., and statistical significance was determined using a Student's *t*-test for pairwise comparisons.

Analysis of NMDAR-mediated sEPSCs. Since virtually all of the spontaneous NMDAR-mediated sEPSCs (NMDAR-sEPSCs) under our recording conditions presented as bursts, and each burst consisted of multiple EPSCs, we used total "charge transfer" as an indicator of the magnitude of NMDAR-sEPSCs; this parameter contained information about both amplitude and frequency (Mouginot et al., 1998). Note that we did not include in the analysis the initial transient component, which represented Na^+ or AMPA-mediated currents depending on the recording conditions and was easily separable temporally from the NMDAR-sEPSCs in each case (see inset figure, below, representing a typical sEPSC). We calculated charge transfer from each individual burst of NMDAR-sEPSCs, starting from the peak of the first NMDAR-sEPSC and continuing to the baseline, which took ~ 5 seconds in the majority of analyzed sEPSCs (hatched area in inset figure, below). Over 800 sEPSCs were analyzed for each condition. For equal temporal epochs, the sum of the charge transferred during individual bursts for each experimental condition (1, 10 or 30 μM memantine, 10 μM ifenprodil, 0.1 μM TTX, 10 μM CNQX, 100 μM NO-711, and each washout period) was normalized to its own baseline value for that same cell. Normalization on a cell-by-cell basis was performed to eliminate experimental error between neurons based on varying dendritic ramification and cell capacitance.



Additionally, we calculated the frequency and amplitude of NMDAR-sEPSC bursts for each condition during ~100 s of recording time (Figure 2.12). The mean amplitude was calculated by averaging the amplitude of the maximal current in each burst of NMDAR-sEPSCs. Each recording was performed under control conditions, in the presence of antagonist, and then during washout. To allow steady-state to be achieved for the analysis, the initial 20 seconds of each drug application or washout were excluded. The data were then normalized for each cell as described above.

Transgenic YAC128 HD mice. Male and female YAC128 mice expressing expanded human huntingtin with 128 CAG repeats and WT littermates maintained on the FVB/N strain (Charles River) were used for these experiments (Slow et al., 2003; Van Raamsdonk et al., 2005). Mice were housed singly or in pairs in duplex cages with littermates of mixed genotype and maintained under a 12 L:12 D light cycle (lights on at 2300) in a clean facility and given free access to food and water. Experimenters were blind to the genotype of the mice. All experiments were performed with the approval of the animal care committee at the University of British Columbia.

Drug treatment. *Dose-finding pilot study*. To determine the treatment doses of memantine, a dose-finding pilot study was conducted in a manner similar to that reported previously (Minkeviciene et al., 2004). Memantine at concentrations of 1, 10, 30, and 100 mg/kg were delivered to animals via the drinking water; the control group received water only. After fours weeks of treatment, blood was obtained by cardiac puncture with heparinized syringes, collected into EDTA-coated tubes, and spun immediately to obtain plasma. Steady-state plasma concentrations of memantine were determined at Merz Pharmaceuticals GmbH (Frankfurt am Main) using a gas chromatograph system coupled with a mass selective detector (Kornhuber and Quack, 1995). Chosen for the trial were a dose of 30 mg/kg, which produces a steady-state plasma level of memantine of about 2 μM (and is further concentrated up to 15-fold in the brain), and a dose that is 30-fold lower, 1 mg/kg (which is known to produce brain levels of ≤1 μM in rodents) (Lipton, 2007).

Memantine treatment. For administration of drugs in the drinking water, water consumption of individual cages was monitored on a biweekly-basis along with animal body weights. The concentrations of drug solutions for each cage were then adjusted accordingly. The drug solutions were replaced twice/week and provided *ad libitum*.

Assessment of motor function. Training and baseline testing for motor function tasks were carried out prior to memantine treatment initiation at 2 months of age during the dark cycle. Assessment of the effect of long-term (10-month) treatment with memantine on motor function was carried out by testing at 12 months of age. Motor coordination and balance were assessed using the accelerating rotarod task (UGO Basile, Comerio, Italy). In this task, the rotarod sped up from 5 revolutions per minute (RPM) to 40 RPM over 4 minutes. Performance in the rotarod

tasks was assessed by the amount of time that a mouse could remain running on the rotarod. During training, mice were given three trials per day for three consecutive days.

Brain sample preparation. Mice were injected with heparin followed by terminal anesthesia with intraperitoneally-injected 2.5% avertin. The animals were perfused with 3% paraformaldehyde with 0.6% glutaraldehyde in phosphate-buffered saline (PBS). Brains were left in the skulls for 24 hrs in 3% paraformaldehyde, then removed and stored in PBS. After weighing, the brains were transferred to a 30% sucrose solution containing 0.08% sodium azide in PBS. They were then frozen on dry ice, mounted with Tissue-TEK O.C.T. compound (Sakura), and sliced coronally into 25 μm sections on a cryostat (Microm HM 500M, Richard-Allan Scientific). The sections were collected and stored in PBS with 0.08% sodium azide at 4 ºC.

Neuropathological analysis. A series of 25 μm-thick coronal sections spaced 200 μm apart spanning the striatum was stained with NeuN antibody (Chemicon) overnight at room temperature, followed by incubation with biotinylated anti-mouse antibody (Vector Laboratories, Burlingame). The signal was amplified with an ABC Elite kit (Vector) and detected with diaminobenzidine (DAB; Pierce). Striatal volumes were determined from a series of mounted sections using StereoInvestigator software (Microbrightfield) by tracing the perimeter of the striatum in serial sections spanning the striatum.

Assessment of huntingtin inclusions in vivo. Perfused brain sections of 25 μm thickness from WT, untreated YAC128, 1 mg/kg- and 30 mg/kg-treated YAC128 animals were immunoassayed with the antibody EM48 to assess the presence of mtHtt inclusions. Polyclonal EM48 antibody was used at 1:500 and DAB was used as the chromogen (Vector). Htt inclusions were defined as EM48-positive staining at the light microscope level. Photographs of mounted sections were

taken on a light microscope (Zeiss) under a 100X objective using a MetaMorph Imaging System (Molecular Devices), and the intensity of the staining of striatal neurons was measured.

Statistical analysis. Data are expressed as mean ± s.e.m. When suitable, results were interpreted using a one-way ANOVA with a Student-Newman-Keuls (SNK) post-hoc test. Pairwise comparisons between genotypes/treatments were assessed with a Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

2.3. Results and discussion

To investigate the relationship between synaptic activity, inclusion formation and neurotoxicity induced by mtHtt, we initially used a neuronal cell culture model of HD in which primary striatal or cortical neurons were transiently transfected with constructs of either full-length or N-terminal Htt encoding wild-type or expanded polyglutamine repeats. Neurons transfected with wtHtt displayed diffuse cytoplasmic expression of huntingtin by immunocytochemistry, while both intranuclear and cytoplasmic/neuropil macroscopic inclusions were present in both striatal (Figure 2.1**a, b**) and cortical neurons (Figure 2.2**a, b**) transfected with mtHtt. Strikingly, when endogenous NMDAR activity was curtailed in these cultures with NMDAR antagonists D-(-)-2 amino-5-phosphonovaleric acid (D-APV), memantine or ifenprodil, we observed a significant decrease in the number of mtHtt-containing inclusions. In contrast, the AMPA (α-amino-3-hydroxy-5-methyl-4-isooxazole propionic acid)-sensitive glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), did not affect inclusion formation. Moreover, neither CNQX nor NMDAR antagonists had any effect on normal huntingtin expression in transfected neurons (Figure 2.3). Hence, the suppressive effect of NMDAR antagonists on mtHtt inclusion formation could not be attributed to a global suppression of protein expression. Importantly, memantine decreased mtHtt-containing inclusions only at high (30 μM) concentrations, but not at lower (≤10 μM) concentrations (Figure 2.1**a, b**; Figure 2.2**a, b**). To mimic more closely the pathophysiological situation, we next utilized neurons transfected with full-length mtHtt bearing a 44Q expansion, or neurons from the striatum of the transgenic YAC128 HD mouse, which has a yeast artificial chromosome (YAC) encoding the entire human HD gene containing 128 CAG repeats (Slow et al., 2003). These models produced similar findings (Figure 2.4).

Unlike other NMDAR antagonists, such as ifenprodil and D-APV, we have shown that memantine is an open-channel blocker, acting via an uncompetitive/fast 'off-rate' mechanism whereby low micromolar concentrations of the drug preferentially inhibit excessive, primarily extrasynaptic, stimulation of NMDARs but relatively preserve physiological synaptic transmission (Lipton, 2007; Lipton, 2006). In contrast, higher concentrations of memantine lose this selectivity and also inhibit normal NMDAR-mediated synaptic activity like other NMDAR antagonists. Thus, blockade of inclusion formation by memantine at higher concentrations, but not lower, most likely results from interrupting endogenous synaptic activity in these cultures. To further characterize the role of synaptic activity in mtHtt aggregation, we used two additional pharmacological tools to suppress normal excitatory synaptic transmission. Both tetrodotoxin (TTX, to block Na^+ channels and thus the propagation of action potentials that lead to endogenous synaptic activity) and NO-711 (to block GABA uptake and hence augment inhibitory neurotransmission) decreased mtHtt-containing inclusions (Figure 2.2**c**). Taken together, our data suggest that interruption of normal excitatory synaptic activity (mediated by NMDARs) ameliorates macro inclusion formation.

To confirm the notion that our pharmacological manipulations modified synaptic activity, we used whole-cell recording with patch electrodes to study spontaneous excitatory postsynaptic currents (sEPSCs). These experiments were designed to analyze the effect of short-term additions of drugs to determine their mechanism of action. The same drugs were used to prevent sEPSCs from triggering the intracellular signaling cascades involved in inclusion formation or neuronal cell death (Figure 2.1; Figure 2.2). We found that total charge transfer during the NMDAR-mediated component of sEPSCs was significantly reduced by TTX or NO-711, in both wtHtt and mtHtt transfected cortical neurons (Figure 2.5**a, b**). Similarly, high (30 μM)

memantine significantly inhibited the NMDAR-mediated component of sEPSCs, while low (1-10 μM) memantine relatively spared this synaptic activity (Figure 2.5**c**). In contrast, ifenprodil (10 μM) blocked this synaptic activity (Figure 2.5**d**). However, in the presence of 10 μM CNQX, NMDAR-mediated synaptic activity was not significantly attenuated (Figure 2.5**e**). Additionally, both memantine (even at low concentrations), and ifenprodil, attenuated extrasynaptic NMDAR-mediated currents (Figure 2.5**f**). The effect of low-dose memantine on extrasynaptic NMDAR-mediated currents was further confirmed by isolating extrasynaptic NMDAR activity using the previously published MK-801 protocol (Hardingham et al., 2002) (Figure 2.6). These electrophysiological findings validate the specificity of our pharmacological tools for NMDARs when studying mtHtt aggregation and neuronal cell death. Next, we examined whether NMDAR-sEPSCs were affected by mtHtt under our conditions. We found that total charge transfer during sEPSCs was not significantly different in mtHtt- vs. wtHtt-transfected neurons (Figure 2.5**g**). Similar findings were observed in striatal slice recordings from the YAC72 mouse model of HD during low-frequency cortical afferent stimulation (Li et al., 2004), simulating the spontaneous activity level in our cultures. At higher levels of stimulation, mtHtt itself is known to enhance NMDAR activity (Zeron et al., 2002).

Next, we investigated the molecular mechanism of mtHtt-induced inclusion formation. Recently, three groups reported in yeast and cell lines that the chaperonin TRiC (TCP1 ring complex, also known as CCT) detoxifies mtHtt, at least in part by forming multiple mtHtt inclusions, while decreasing toxic soluble microaggregates of mtHtt and thereby decreasing toxicity (Pickett, 2006). Augmentation of TRiC activity increased the appearance of multiple, small mtHtt-containing inclusions (Figure 4.3c and 5c in Tam et al. (Tam et al., 2006)), reminiscent of our findings in neurons with preserved synaptic activity (our Figure 2.2**a**; Figure 2.1**a, 2**). Hence, we

examined if synaptic activity regulates the expression of TCP1, the subunit of TRiC that preferentially interacts with mtHtt (Tam et al., 2006). We added TTX to our neuronal cultures to block synaptic activity and found that it significantly reduced the expression of TCP1 (Figure 2.2**d**). While low-dose memantine (10 μM) did not affect the TCP1 expression, both high-dose memantine (30 μM) and ifenprodil also decreased TCP1 levels (Figure 2.7), supporting the notion that synaptic NMDAR activity is necessary for TCP1 expression. To test if TCP1 is involved in synaptic activity-induced inclusion formation, we generated two small hairpin RNA interference constructs (shRNAs designated TCP1 RNAi-1 and RNAi-2) to knockdown TCP1 expression. TCP1 RNAi-1 and RNAi-2 reduced TCP1 levels to 70% and 60%, respectively (Figure 2.8). Both small hairpin TCP1 RNAi vectors significantly decreased inclusion formation from mtHtt (Figure 2.2**e**). Additionally, knockdown of TCP1 by RNAi occluded the inhibitory effect on inclusion formation exerted by suppression of synaptic activity with high-dose (30 μM) memantine (Figure 2.9). Taken together, our results indicate that synaptic activity induces expression of TCP1, which in turn mediates inclusion formation in neurons. Moreover, TRiC has been reported to cooperate with the Hsp70 chaperone system (Behrends et al., 2006; Kitamura et al., 2006; Melville et al., 2003); Hsp70 attaches to mtHtt inclusions to protect cells from death (King et al., 2008; Kitamura et al., 2006). Indeed, here we observed co-localization of inclusions and Hsp70 (Figure 2.10), suggesting that the TRiC-Hsp70 system might ameliorate mtHtt toxicity.

We have thus shown that physiological synaptic NMDAR activity is necessary for macro inclusion formation in mtHtt-expressing neurons. However, the relationship of mtHtt-induced inclusion formation and neurotoxicity in HD pathogenesis has remained contentious (Davies et al., 1997; Saudou et al., 1998). Large aggregates of abnormally folded proteins have been

hypothesized to contribute to synaptic damage in neurodegenerative disorders (Tsai et al., 2004), but a recent report suggests that inclusion formation protects neurons from cell death (Arrasate et al., 2004), possibly by decreasing the level of toxic soluble forms of mtHtt (Sánchez et al., 2003). Neurodegeneration in HD has also been proposed to represent excitotoxic-mediated apoptotic cell death triggered by excessive activation of NMDARs (Friedlander, 2003). Increased sensitivity to NMDAR-mediated excitotoxicity has been observed in the YAC128 mouse model of HD (Zeron et al., 2002). On the other hand, recent studies have indicated that physiological levels of synaptic NMDAR activity (predominantly composed of NR2A subunits in mature neurons) promote survival in the face of various forms of stress (Hardingham et al., 2002). The exact role of normal neuronal electrical activity in the pathogenesis of HD has not previously been studied.

Our observation that endogenous synaptic NMDAR activity promotes formation of mtHtt-containing inclusions prompted our investigation of whether this normal activity could protect neurons. Conversely, we hypothesized that excessive activation of extrasynaptic NMDARs may increase neuronal vulnerability induced by mtHtt. To test this notion, we initially applied a relatively low concentration of exogenous glutamate (50 μM), which by itself was not excitotoxic to untransfected or wtHtt-transfected neurons under these conditions, to stimulate both extrasynaptic and synaptic receptors. While transfection of mtHtt by itself did not result in neuronal death, as shown previously (Zeron et al., 2002), we found that mtHtt neurons became more vulnerable in the presence of 50 μM glutamate (Figure 2.11**a-c**). When mtHtt neurons were treated with memantine or ifenprodil, neuronal cell death induced by exogenous glutamate was significantly reduced. Of note, we found that low concentrations of memantine (1-10 μM), which selectively block excessive extrasynaptic NMDAR activity while sparing physiological

synaptic NMDARs (Figure 2.5; Figure 2.6, Figure 2.12) (Lipton, 2007; Lipton, 2006), were sufficient to protect mtHtt-transfected neurons from glutamate challenge. Additionally, extrasynaptic NMDARs preferentially contain NR2B subunits (Tovar and Westbrook, 1999), and ifenprodil, a relatively selective inhibitor of NR2B, blocked this neurotoxicity (Hardingham et al., 2002; Zeron et al., 2002). Taken together, these results imply that mtHtt renders neurons more vulnerable to acute excitotoxic insults and that blockade of extrasynaptic NMDARs efficiently ameliorates this excitotoxic cell death. In this regard, we observed similar results for both truncated (N-terminal fragment) (Figure 2.11**b**) and full-length Htt constructs (Figure 2.11**c**).

Next, we examined the effect of normal synaptic activity on survival of neurons expressing mtHtt. We found that TTX, which suppresses normal excitatory synaptic transmission, or NO-711, which enhances inhibitory neurotransmission, triggered significant cell death in mtHtt- but not wtHtt-expressing neurons (Figure 2.11**d**; Figure 2.13). We then asked if extrasynaptic NMDAR activity causes cell death in mtHtt neurons when protective (physiological) synaptic NMDAR activity is inhibited. Accordingly, we investigated if an NMDAR antagonist that preferentially blocks extrasynaptic activity could protect mtHtt neurons from toxicity induced by TTX or NO-711. Indeed, we found that treating mtHtt cultures with low concentrations (~5 μM) of memantine significantly reduced neuronal death induced by TTX or NO-711 (Figure 2.11**d**). The question arises where the extrasynaptic glutamate originates in our culture system in the presence of neuronal activity blockade with TTX because synaptic release is inhibited. Since the intracellular pool of glutamate, used for metabolic purposes, may be significant, up to 10 mM per cell (Lipton and Rosenberg, 1994), mtHtt-insulted neurons may potentially leak glutamate from nonsynaptic sites. Additionally, similar to the intact brain, our culture system contains nonneuronal cells, predominantly astrocytes. Even in the absence of synaptic activity, glutamate

released from astrocytes can activate extrasynaptic NMDARs in neurodegenerative conditions (Fellin et al., 2004; Tian et al., 2005).

We next wanted to investigate the relationship of macro inclusion formation, electrical activity, and neuronal survival. Since we had demonstrated earlier that TCP1 mediates inclusion formation (Figure 2.2**e**), we examined if TCP1 is involved in the survival of neurons expressing mtHtt. We found that knockdown of TCP1 not only decreased inclusion formation but also significantly increased neuronal cell death in mtHtt-expressing neurons (Figure 2.11**e**). These RNAi experiments imply causality between TCP1 levels, inclusion formation, and neuronal cell death. Importantly, this form of cell death was significantly ameliorated by blockade of extrasynaptic NMDAR using low-dose memantine (Figure 2.11**e**). Taken together, our results suggest that synaptic activity induces expression of TCP1, which in turn contributes to protective inclusion formation and decreased neurotoxicity. Conversely, blockade of synaptic activity reduces TCP1 levels, which in turn decreases mtHtt inclusion formation, and contributes to neuronal cell death in conjunction with excessive extrasynaptic NMDAR activity.

Furthermore, CREB/CBP transcriptional activity, which triggers the neuroprotective PGC-1α pathway, has been shown to be decreased by extrasynaptic NMDAR activity (Hardingham et al., 2002) as well as by binding to soluble mtHtt (Nucifora et al., 2001). We therefore hypothesized that impairment of the CREB—PGC-1α cascade may contribute to neuronal cell death in HD (McGill and Beal, 2006). Hence, we determined if CREB function was compromised in mtHtt-expressing neurons when synaptic activity was blocked. In mtHtt- but not wtHtt-transfected neurons we found a significant decrease in CREB activity in the presence of TTX, which suppresses excitatory synaptic activity (Figure 2.11**f**). Additionally, blockade of extrasynaptic NMDARs with low concentrations of memantine restored CREB function,

suggesting that mtHtt and activation of extrasynaptic NMDARs are necessary for CREB inactivation. We next determined the level of PGC-1α under these same conditions. We observed a significant decrease in PGC-1α levels in mtHtt-transfected neurons in the presence of TTX, but preservation of PGC-1α levels in the presence of low concentrations of memantine (Figure 2.11**g**; Figure 2.14). These findings support the notion that mtHtt and excessive extrasynaptic NMDAR activity interfere with the neuroprotective CREB—PGC-1α cascade. Importantly, decreased levels of PGC-1α likely contribute to cell death in mtHtt-transfected neurons exposed to TTX (and thus in neurons with blocked synaptic activity and decreased mtHtt inclusions). As would be predicted, cell death under these conditions was diminished by co-transfection with PGC-1α (Figure 2.11**d**).

These findings in conjunction with the recent demonstration of increased extrasynaptic NMDAR expression and excessive activity in the YAC128 HD mice (Milnerwood et al., 2010) raise the possibility that long-term blockade of extrasynaptic activity would be advantageous while simultaneous blockade of synaptic activity might prove deleterious in vivo, for example, in the transgenic YAC128 HD mouse (Slow et al., 2003) (Figure 2.4**b**). We hypothesized that the balance between synaptic and extrasynaptic activity in the face of mtHtt would determine neuronal survival. Therefore, while short-term blockade of both synaptic and extrasynaptic activity might cause transient improvement, eventually blockade of synaptic activity would lead to neuronal loss. In contrast, maintenance of synaptic activity with abrogation of excessive extrasynaptic activity would be most beneficial to combat the deleterious effects of mtHtt. To test this hypothesis in vivo, YAC128 mice were treated using drinking water with low-dose memantine (1 mg/kg) to block extrasynaptic NMDAR activity or high-dose memantine (30 mg/kg) to additionally block synaptic NMDAR activity since we knew that these doses would

produce low and high concentrations of memantine approaching those used in the culture experiments (Chen et al., 1992; Hesselink et al., 1999; Lipton, 2007; Lipton, 2006; Parsons et al., 2007). Treatment commenced at 2 months of age and continued for 10 months until 12 months of age. We first analyzed mtHtt inclusions using immunostaining with antibody EM48, which recognizes N-terminal huntingtin and is highly specific for aggregates (Gutekunst et al., 1999). Treatment with low-dose memantine increased inclusion formation (Figure 2.15), as confirmed using a filter trap assay (Figure 2.16), while high-dose memantine significantly decreased inclusion formation in 12-month-old YAC128 mice (Figure 2.15). These effects of memantine treatment on inclusion formation were not due to alterations in mtHtt protein expression since low and high doses of memantine did not affect mtHtt levels (Figure 2.17). On the other hand, TCP1 levels increased with low-dose memantine and decreased with high-dose memantine (Figure 2.18), supporting the notion that normal synaptic NMDAR activity increases TCP1 and promotes inclusion formation.

Next, we measured loss in striatal volume in YAC128 mice, a cardinal neuropathological feature of HD (Vonsattel et al., 1985). Low-dose memantine treatment in YAC128 animals improved striatal volume, while high-dose memantine worsened this parameter (Figure 2.19**a**). Similarly, treatment of WT animals with low-dose memantine led to a significant increase in striatal volume, while high-dose memantine led to a significant decrease in striatal volume (Figure 2.19**b**). These findings support our hypothesis that maintenance of synaptic activity, while inhibiting excessive extrasynaptic activity, is protective, and that long-term blockade of synaptic activity is detrimental. We also monitored motor function by rotatrod testing. YAC128 HD mice treated with low-dose memantine demonstrated improvements on these motor tests, while high-dose-treated mice showed no improvement (Figure 2.20**a**). Specifically, YAC128 HD mice

treated with low-dose, but not high-dose, memantine showed significant improvement at 12, but not 6, months of age (Figure 2.20**b**). Similarly, WT animals treated with low-dose memantine showed significant improvement in motor rotarod performance at 6, 8, and 12 months of age, while high-dose treated mice showed no improvement (Figure 2.20**c, d**). Taken together, these results support the premise that maintenance of synaptic activity with abrogation of extrasynaptic activity is beneficial in YAC128 HD mice.

Our results provide a mechanistic framework to help us understand the selective vulnerability of striatal and cortical neurons in HD. We show here that synaptic activity controls expression of the chaperonin TRiC, which in turn modulates inclusion formation and toxicity of mtHtt. Moreover, the electrical properties of these neurons coupled with the predominant effect of the CREB—PGC1α pathway in this cell type (Cui et al., 2006) renders them particularly sensitive to the toxic effects of mtHtt. Specifically, excessive extrasynaptic activity and mtHtt decrease CREB—PGC-α activity to promote neuronal cell death. Coupled with a decrease in synaptic transmission, which also contributes to a decrease in CREB activity in the presence of mtHtt, neurons become increasingly vulnerable to injury and death (Figure 2.21). We speculate that these findings imply that therapies for HD that minimize excessive extrasynaptic activity while maintaining or enhancing normal synaptic activity will yield considerable benefit. Notably, it has been contentious whether mutant huntingtin by itself is cytotoxic in cell culture. Along these lines, it has been demonstrated that mtHtt contributes to neuronal cell death by increasing excitotoxic vulnerability (Zeron et al., 2002). We report similar findings here, and, in fact, our data using primary neurons manifesting synaptic activity may resolve the question of direct mtHtt toxicity raised by the prior findings of others using yeast, cell lines, or synaptically-immature neurons. We now show not only that mtHtt increases the vulnerability of neurons to

relatively low concentrations of exogenous glutamate, but also that suppressing spontaneous excitatory synaptic activity can mimic this phenomenon via the excitotoxic effect of endogenous glutamate apparently acting on extrasynaptic receptors.

Our findings further suggest that the balance between synaptic and extrasynaptic NMDAR activity may be critical in determining neuronal cell survival in HD. We show that low concentrations of the NMDAR antagonist, memantine, afford the advantage of restoring excitatory balance by maintaining physiological synaptic activity while blocking excessive extrasynaptic NMDAR stimulation (Lipton, 2007; Lipton, 2006). Thus, we posit that restoring excitatory balance, which is disrupted by mtHtt protein, can affect protein misfolding and protect neurons in HD, as also suggested in a small, open-label human clinical trial of memantine in HD patients (Beister et al., 2004). This novel concept of balancing synaptic and extrasynaptic neuronal NMDAR activity may also lead to strategies to combat cell injury and death associated with other neurodegenerative disorders.

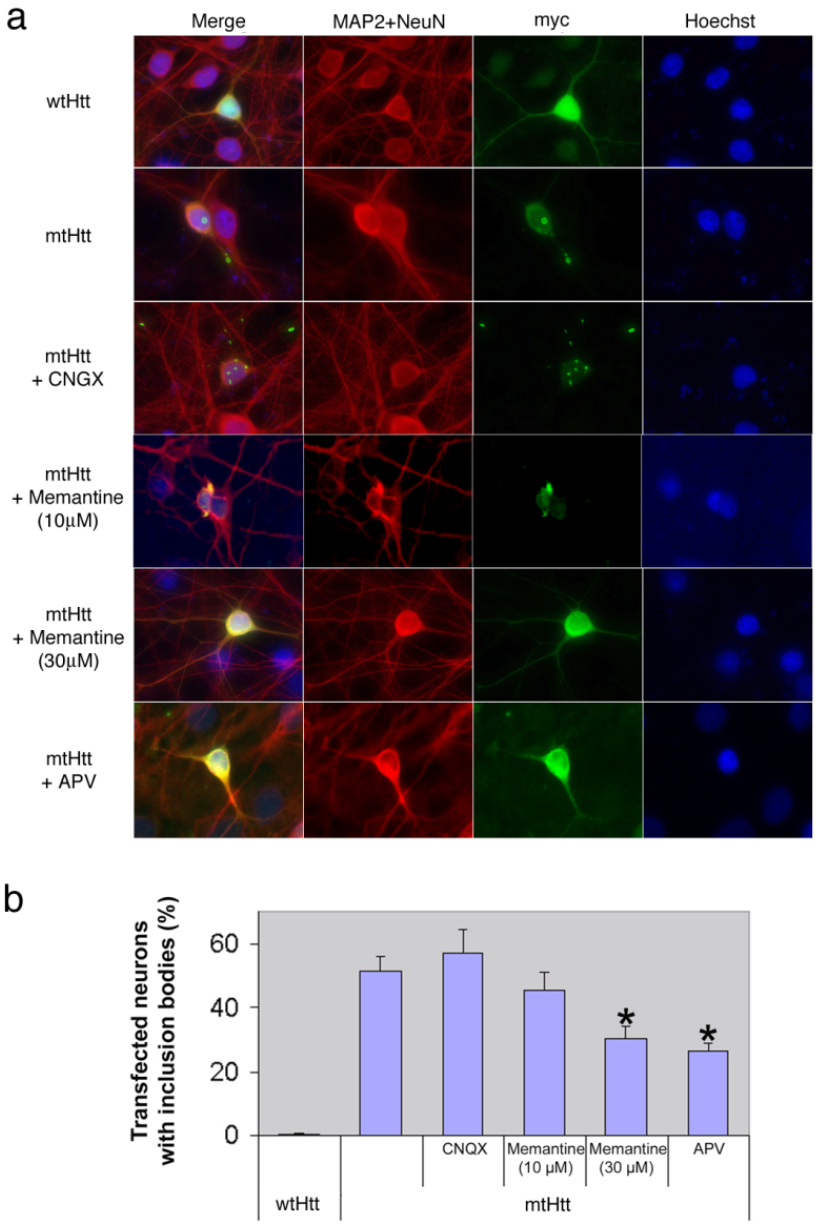


Figure 2.1 Inclusion formation in mtHtt-expressing striatal neurons is ameliorated by NMDAR antagonists

(a) Rat primary striatal neurons were transfected with wtHtt (Myc-mtHtt-N63-18Q) or mtHtt (Myc-mtHtt-N63-148Q). Similar to cortical neurons, wtHtt-expressing striatal neurons displayed diffuse cytoplasmic labeling, while mtHtt formed inclusions in both nuclei and neurites. The AMPA receptor antagonist CNQX (10μM) or low-dose NMDAR antagonist memantine (10μM) manifested no effect on inclusions formed from mtHtt. High-dose memantine (30 μM) or d-APV (150 μM) significantly reduced the number of inclusions in mtHtt-transfected neurons. (b) Quantification of blockade of mtHtt-inclusions in transfected striatal neurons. CNQX (10 μM) or low concentrations of memantine (10 mM) did not inhibit inclusion formation. Values are mean ± s.e.m. for n ≥ 250. *, P < 0.01 by ANOVA.

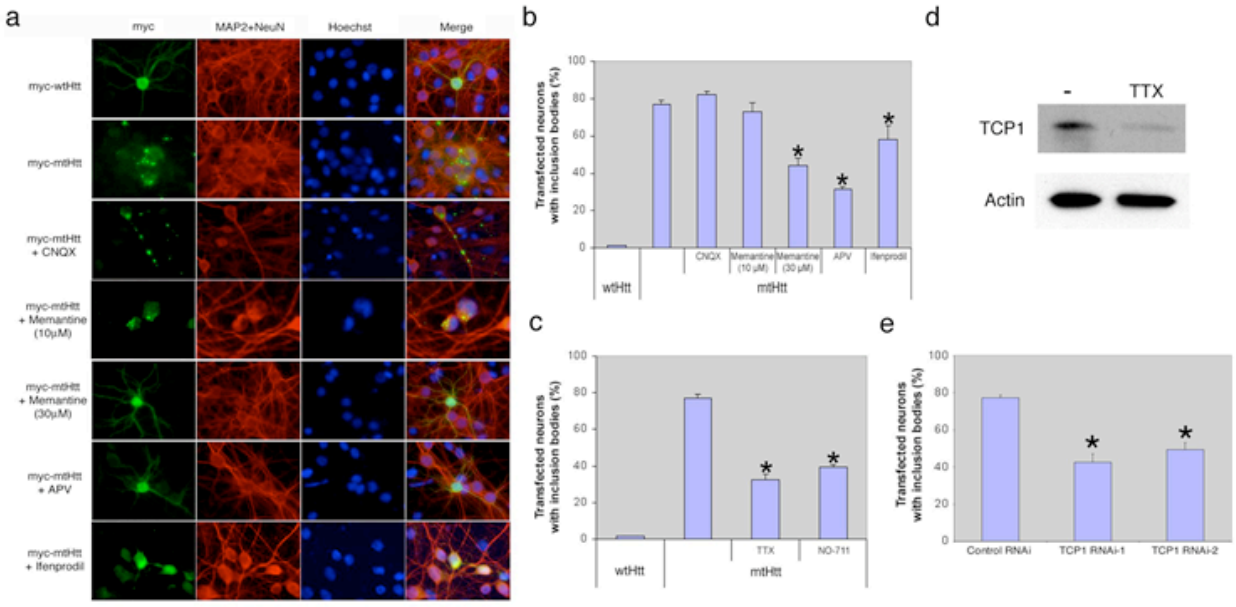


Figure 2.2 Suppression of excitatory NMDAR synaptic transmission ameliorates inclusion formation in mtHtt-expressing neurons

(**a, b**) Rat primary cortical neurons were transfected with wtHtt (Myc-mtHtt-N63-18Q) or mtHtt (Myc-mtHtt-N63-148Q). WtHtt-expressing neurons displayed diffuse cytoplasmic labeling while mtHtt formed inclusions in both nuclei and neurites. AMPA receptor antagonist CNQX manifested no effect on inclusions formed from mtHtt. NMDAR antagonists memantine (30 μM) and D-APV (150 μM) significantly reduced the number of inclusions in mtHtt-transfected neurons. NMDAR antagonists significantly decreased inclusion formation in mtHtt neurons. Although not quite as effective, 10 μM ifenprodil, a relatively selective inhibitor of NR2B subunits of the NMDAR manifested a similar action. In contrast, CNQX or low concentrations of memantine (1-10 μM) did not inhibit inclusion formation. Values are mean ± s.e.m. ($n \geq 1{,}200$). *, $P < 0.01$ by ANOVA. (**c**) Rat primary neurons were transfected with wtHtt or mtHtt and treated with synaptic activity suppressors (0.2 μM TTX or 100 μM NO-711). Inclusion formation was quantified. Values are mean ± s.e.m. *, $P < 0.01$ by ANOVA. (**d**) Neurons were exposed to TTX (0.2 μM) or control conditions. After 24-h incubation, total cell extracts were prepared. Immunoblotting demonstrated that TTX reduced the level of TCP1 protein expression. Actin served as a loading control. (**e**) Neurons were transfected with mtHtt plus two different small hairpin vectors for TCP1 or control vector. Inclusion formation was quantified. TCP1-knockdown attenuated inclusion formation. Values are mean ± s.e.m. for $n \geq 300$. *, $P \leq 0.001$ by ANOVA.

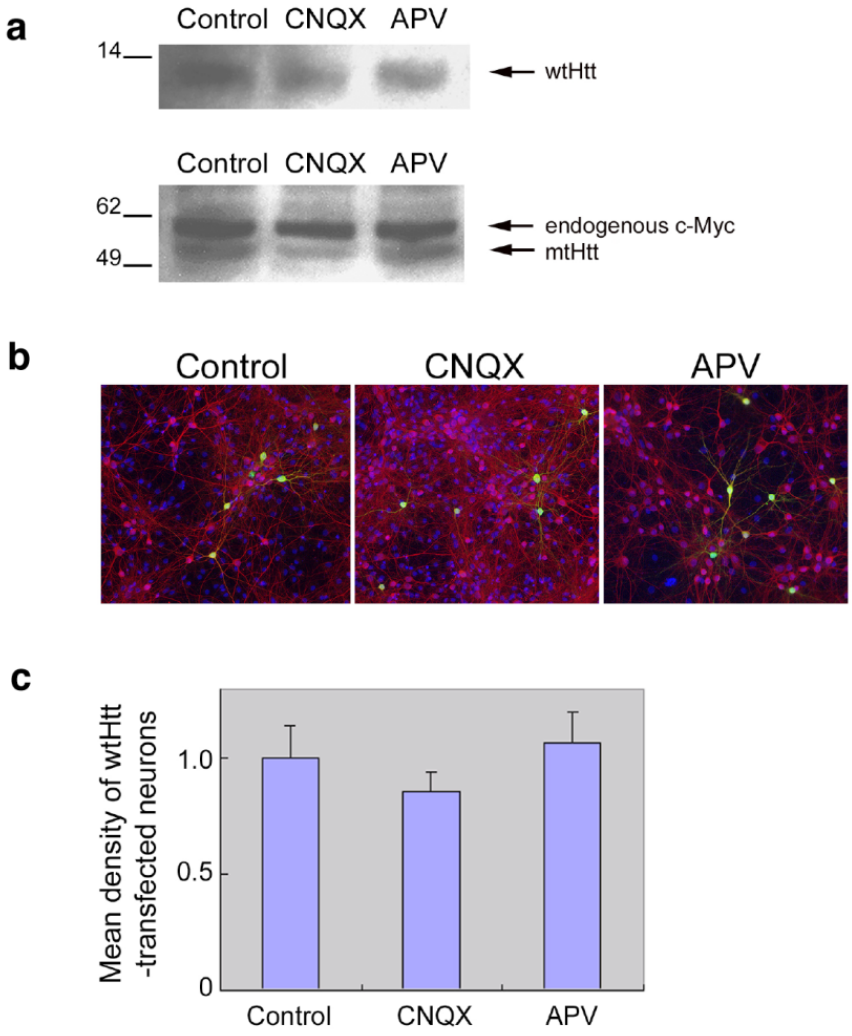


Figure 2.3 Htt expression is unaffected by d-APV or CNQX

(a) Rat primary cortical neurons were transfected with either wtHtt (Myc-mtHtt-N63-18Q) or mtHtt (Myc-mtHtt-N63-148Q). Cell lysates were subjected to Western blot analysis with anti-Myc antibody (Santa Cruz) to detect expression of huntingtin protein. Application of d-APV (150 μM) or CNQX (10 μM) did not change the expression levels of wtHtt or mtHtt. (b, c) wtHtt-transfected cortical neurons were stained with anti-Myc antibody (endogenous Myc is not detected under our staining conditions), and the expression of Htt protein was quantified by measuring mean fluorescence intensity under deconvolution microscopy. There was no significant difference in Htt expression among control, d-APV- and CNQX-treated neurons (by ANOVA).

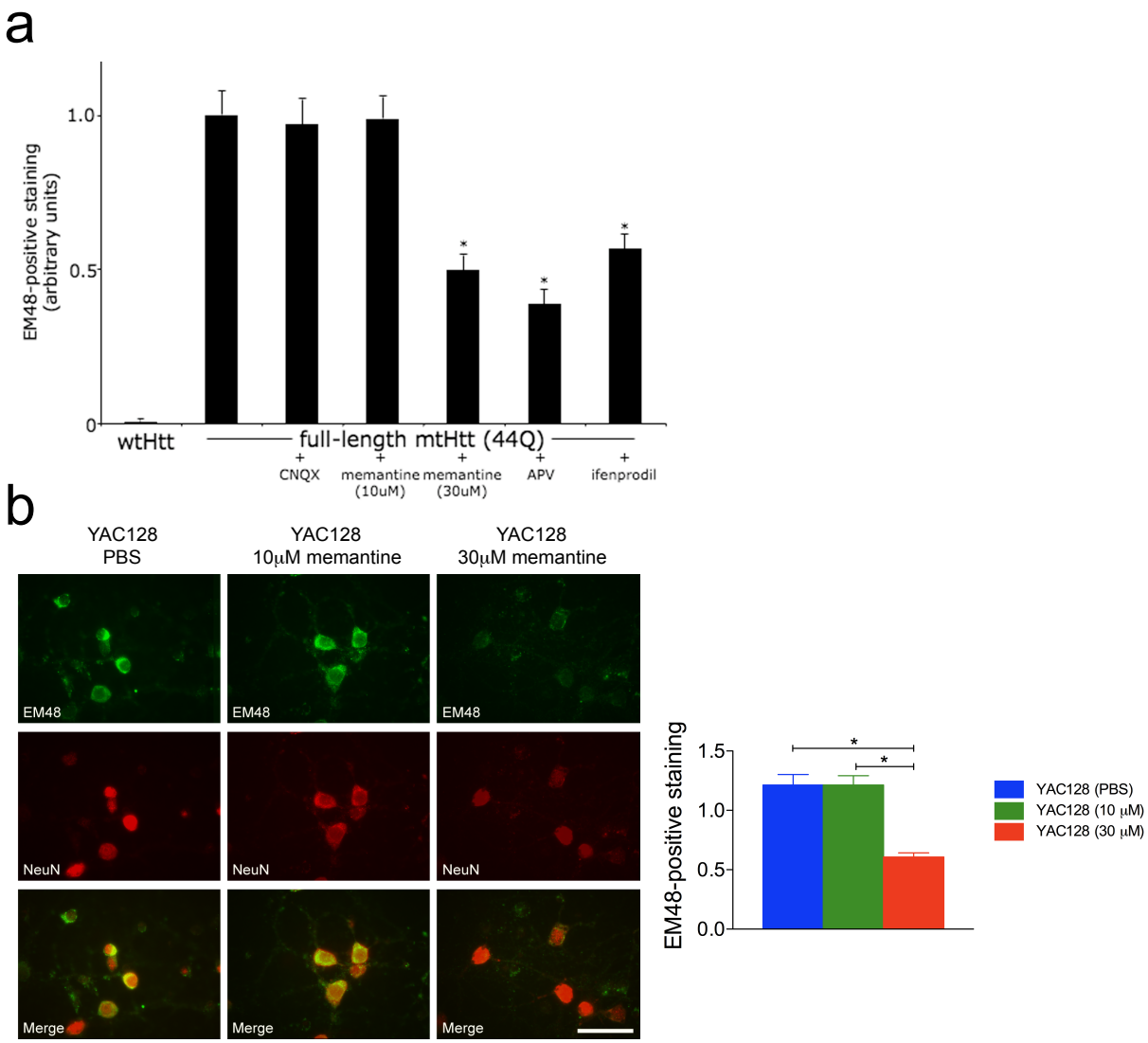


Figure 2.4 Effects of NMDAR antagonists on inclusion formation in neurons expressing full-length mtHtt with 44Q and striatal neurons from YAC128 HD animals

(a) Rat primary neurons were transfected with full-length wtHtt or full-length mtHtt with 44Q. The extent of inclusion formation was assessed using unbaised intensity measurements of striatal neurons stained with EM48. The NMDAR antagonists memantine (30 μM), d-APV (150 μM), and ifenprodil (10 μM) significantly reduced the number of inclusions. The AMPA receptor antagonist CNQX or low concentrations of memantine (10 μM) did not inhibit inclusion formation. (b) Primary striatal neurons were dissected from P0 YAC128 pups and cultured for 9 days. On day 7, neurons were treated with either vehicle, low-dose (≤10 μM) or high-dose (30 μM) memantine for 48 h. Quantification of the extent of EM48 staining revealed significantly lower levels in neurons treated with 30 μM memantine compared to vehicle- or 10 μM memantine-treated neurons. Values are mean ± s.e.m. $P < 0.01$ by ANOVA.

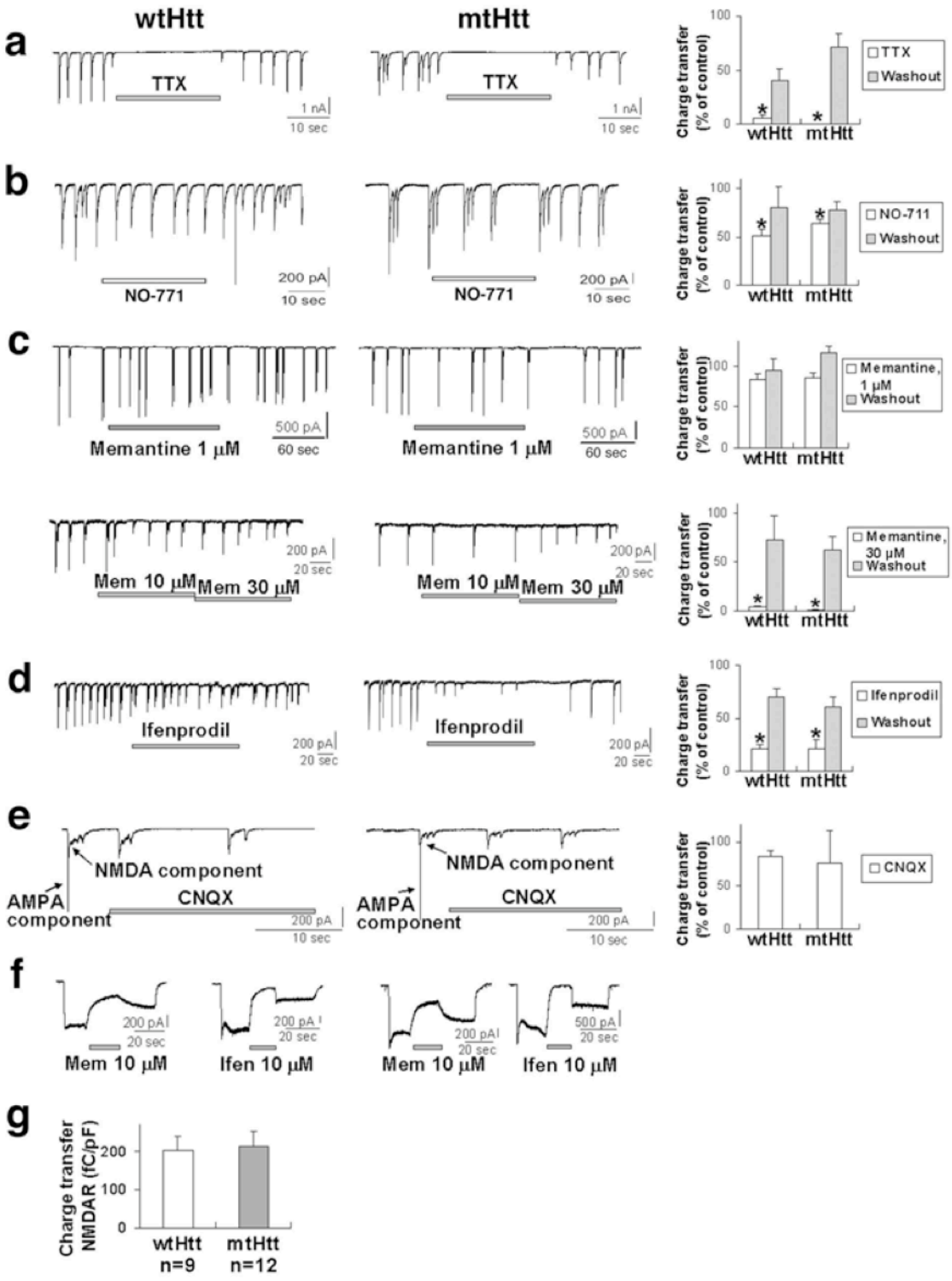


Figure 2.5 Pharmacology of NMDAR-mediated sEPSCs and whole-cell currents recorded from wtHtt- and mtHtt-transfected neurons

(**a-e**) sEPSCs (*left-hand panels*) and charge transfer (*right-hand panels*), normalized for each cell. NMDAR-mediated sEPSCs in both wtHtt- and mtHtt-transfected neurons were significantly inhibited by TTX (**a**), NO-711 (**b**), ifenprodil (**d**), and 30 μM memantine (**c**), but was relatively spared by 1-10 μM memantine (**c**) or CNQX (**e**). Recording solutions in (**a-e**) contained 20 μM glycine; for (**c. d**), CNQX and bicuculline (10 μM each) were also added. Values are mean ± s.e.m. ($n \geq 4$ cells in each case); *, $P < 0.03$ (paired *t*-test on raw data). (**f**) Both memantine and ifenprodil attenuated extrasynaptic NMDAR-mediated currents. Incomplete reversal was observed because of slow washout in this system. Recordings were performed in the presence of 0.1-1 μM TTX. NMDA currents were evoked by co-application of 100 μM NMDA and 20 μM glycine. (**g**) For NMDAR-sEPSCs, charge transfer (fC/pF) was not significantly different between mtHtt- and wtHtt-transfected neurons.

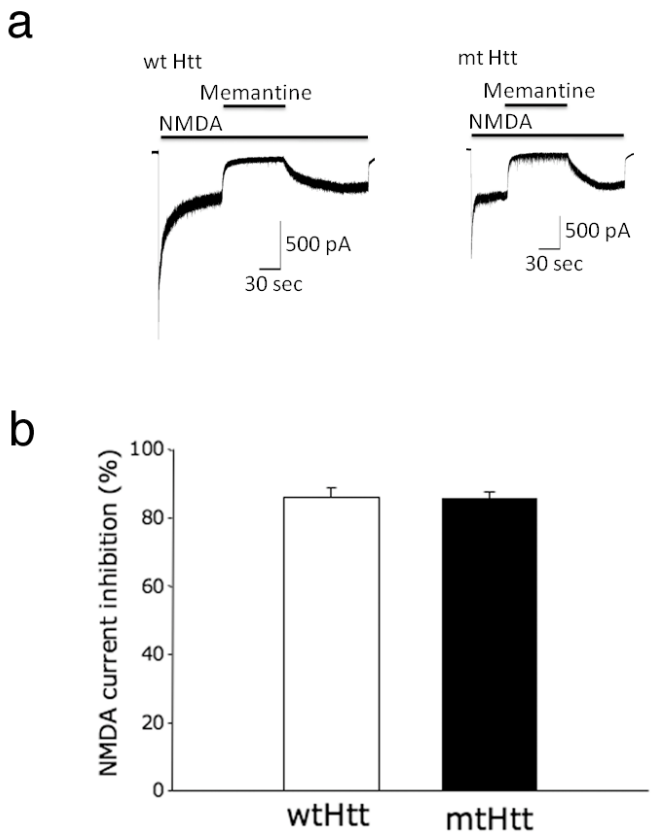


Figure 2.6 Low-dose memantine blocks extrasynaptic NMDARs in both wtHtt- and mtHtt-transfected neurons

To isolate extrasynaptic NMDAR activity, synaptic NMDA receptors were selectively inactivated using 10 μM MK-801 in conjunction with 50 μM bicuculline, while extrasynaptic NMDARs were spared, as described previously11. Extrasynaptic NMDAR activity was evoked by bath application of 100 μM NMDA plus 10 μM glycine. (a) Inhibitory effect of 10 μM memantine on NMDA-induced extrasynaptic currents in wtHtt-transfected (left) and mtHtt-transfected neurons (right). (b) Memantine blocked >85% of the extrasynaptic NMDAR current in both wild-type huntingtin-transfected neurons (85.9 ± 3.0%) and mutant huntingtin-transfected neurons (85.7 ± 2.0%).Values are mean ± s.e.m.; n = 5 for each group.

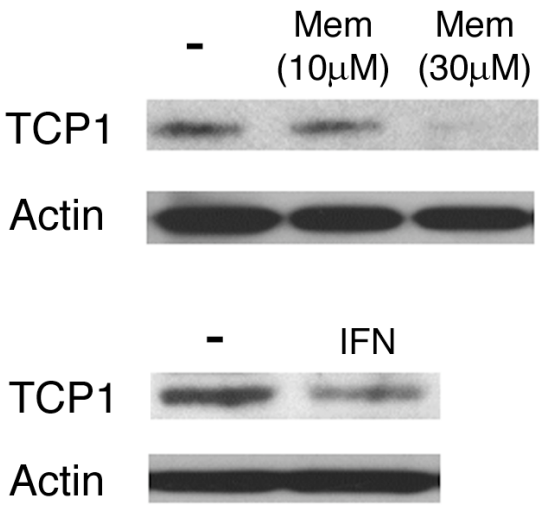


Figure 2.7 Effect of NMDAR antagonists on expression of the TRiC chaperonin subunit TCP1

Neurons were exposed to memantine (Mem) or ifenprodil (IFN; 10μM). After a 24-h incubation, total cell extracts were prepared. Immunoblotting demonstrates that high-dose memantine or ifenprodil reduced the level of TCP1 protein expression. The moderate effect of ifenprodil on TCP1 levels parallels the moderate effect of ifenprodil on inclusion formation (Figure 4.1b). Actin served as a loading control.

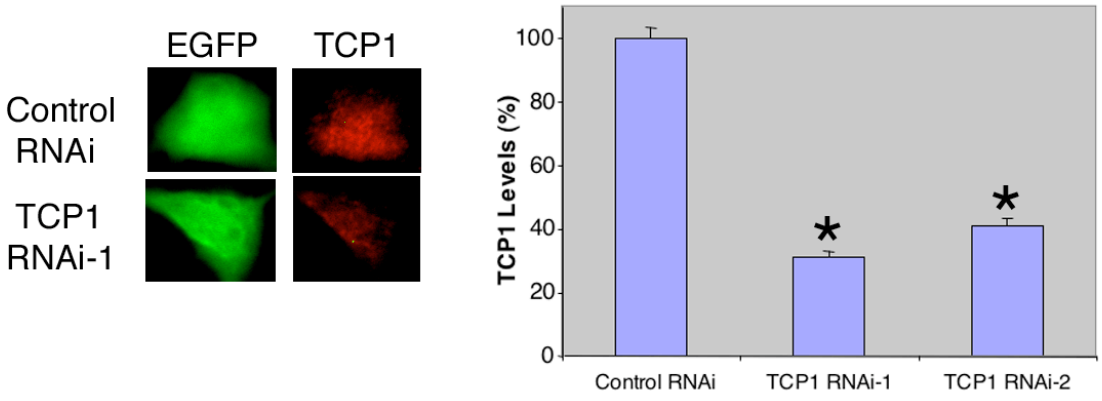


Figure 2.8 Knockdown of TCP1 in neurons using small hairpin expression vectors

Images of neurons transfected with the indicated shRNA expression plasmid, identified by co-expression of EGFP (green), and stained with anti-TCP1 antibody (red). Relative TCP1 levels were quantified in transfected neurons by deconvolution fluorescence microscopy. Values represent mean ± s.e.m. for n ≥ 300. *, P < 0.001 by ANOVA.

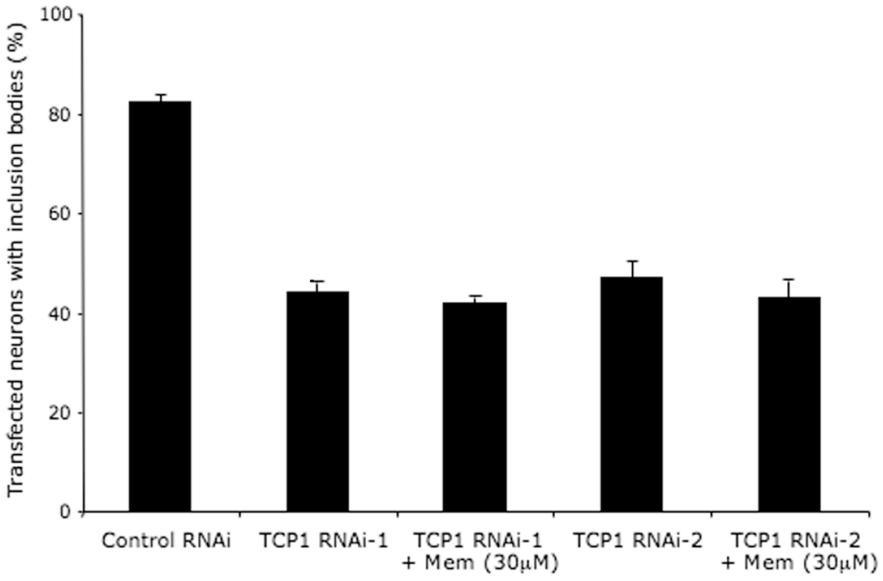


Figure 2.9 Effect of high-dose memantine (30 μM) on inclusion formation in neurons in which TCP1 had been knocked down by RNAi

Neurons were transfected with mtHtt plus two different small hairpin vectors for TCP1 or control vector, and treated with 30 μM memantine. TCP1-knockdown attenuated inclusion formation. Values are mean ± s.e.m.

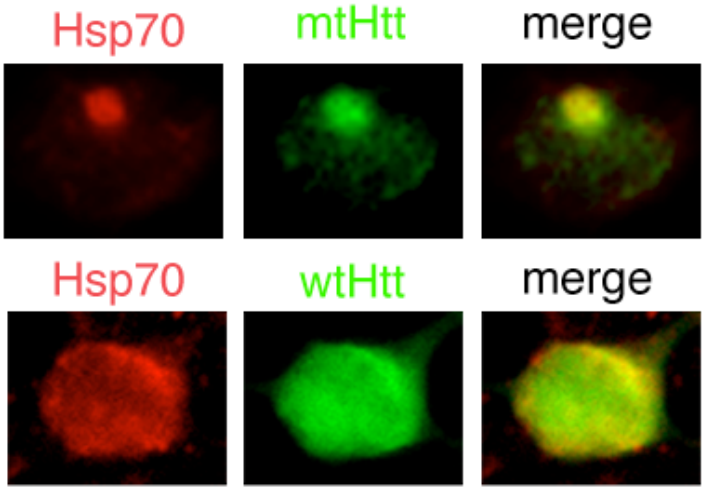


Figure 2.10 HSP70 co-localizes with mtHtt inclusion

Neurons were transfected with myc-tagged mtHtt or myc-tagged wtHtt. Distribution was examined using immunostaining for Hsp70 and myc.

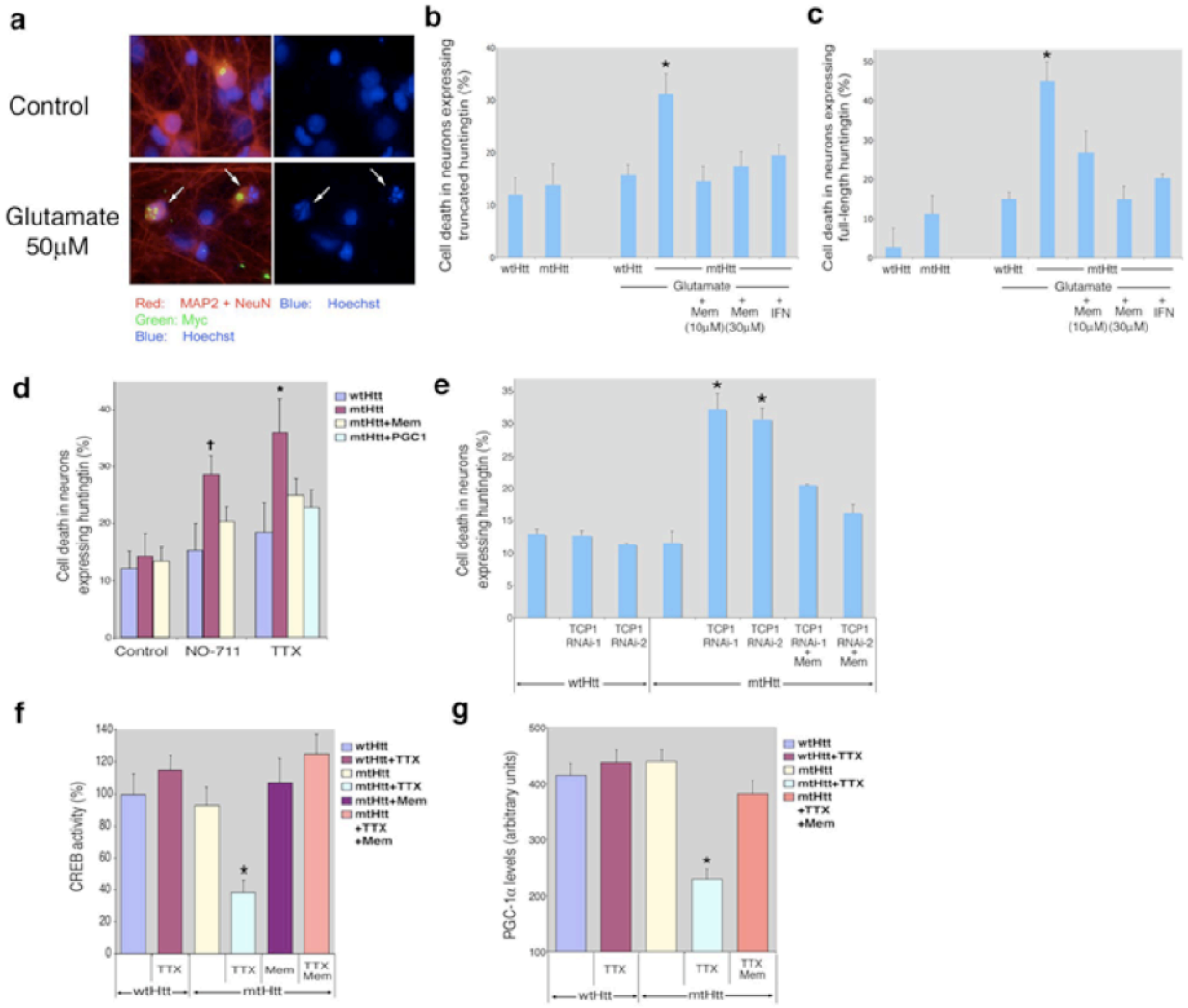


Figure 2.11 Excitatory synaptic versus extrasynaptic activity in HD-related neuronal cell death

(**a**) Excessive glutamate insult led to neuronal cell death in mtHtt-transfected cortical neurons in the presence of inclusion formation. Neurons transfected with mtHtt contained inclusions and displayed healthy nuclei. After incubation in 50 μM glutamate for 40 h, significant cell death occurred in mtHtt-transfected neurons in the presence of inclusion formation. (**b, c**) Cell death analysis after transfection with the N-terminal fragment of mtHtt (**b**) and full-length mtHtt (**c**). In the absence of exogenous glutamate, cell death did not vary between wtHtt- and mtHtt-transfected neurons. Glutamate at 50 μM induced significant neuronal loss in mtHtt but not wtHtt neurons. Memantine (10 and 30 μM) protected mtHtt-transfected neurons from glutamate excitotoxicity. Ifenprodil manifested a similar protective effect. Values represent mean ± s.e.m. for $n \geq 1{,}200$. *, $P < 0.01$ by ANOVA. (**d**) Memantine attenuated neuronal cell death induced by blockade of physiological excitatory synaptic activity with NO-711 or TTX in mtHtt-transfected neurons. Both striatal and cortical cultures were used in these experiments with similar results. Either NO-711 (100 μM) or TTX (0.2 μM) induced significant neuronal loss in mtHtt- but not wtHtt-transfected neurons. Memantine prevented this neurotoxicity. Co-transfection of PGC-1a mitigated neuronal cell death induced by blockade of synaptic activity with TTX. Values represent mean ±

s.e.m. for $n \geq 1{,}200$. *, $P < 0.01$; †, $P < 0.05$ by ANOVA. (**e**) Neurons were transfected with wtHtt or mtHtt plus two different small hairpin vectors for TCP1 or a control vector. Neuronal cell death was quantified as described in the Methods section. TCP1-knockdown induced death in mtHtt-expressing neurons but was ameliorated with a relatively low concentration of memantine (Mem, 5 μM). Values are mean ± s.e.m. for $n \geq 300$. *, $P \leq 0.01$ by ANOVA. (**f**) Low concentration (5 μM) memantine abrogated the decrease in CREB activity induced by blockade of physiological excitatory synaptic activity with TTX in mtHtt-transfected neurons. Neurons were transfected with a CRE-luciferase reporter construct and wtHtt or mtHtt. Ordinate axis represents relative luciferase activity. Values represent mean ± s.e.m. *, $P < 0.01$ by ANOVA. (**g**) Low concentration memantine ameliorated the decrease in PGC-α levels induced by blockade of synaptic activity with TTX in mtHtt-transfected neurons. PGC-1α levels were quantified by immunofluorescence under deconvolution microscopy in wtHtt- or mtHtt-transfected neurons. Values represent mean ± s.e.m. for $n \geq 300$. *, $P < 0.01$ by ANOVA.

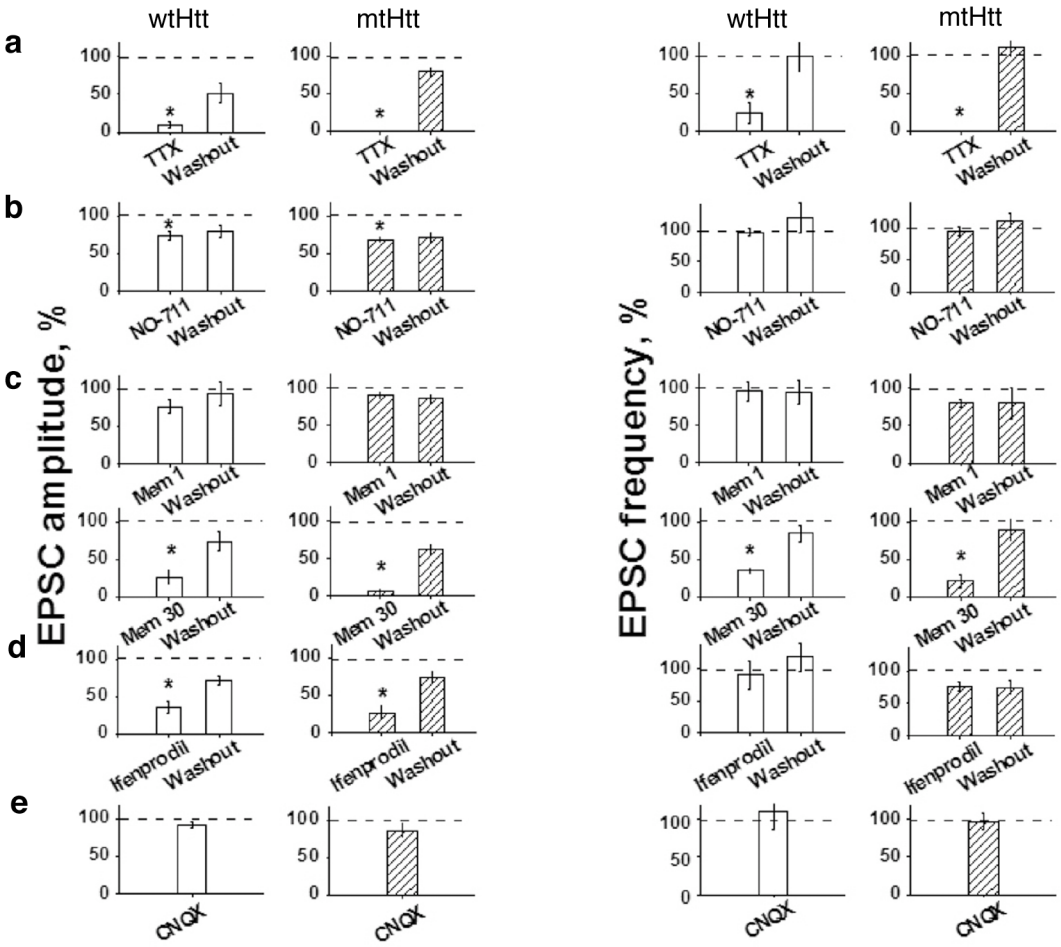


Figure 2.12 Analysis of normalized amplitude and frequency of NMDAR-mediated sEPSCs

Amplitude (left) of NMDAR-mediated sEPSCs in both wtHtt (Htt-N63-18Q) and mtHtt (Htt-N63-148Q) transfected neurons was significantly inhibited by 0.1 μM TTX (a), 100 mM NO-711 (b), 30 μM memantine (c, bottom panel), and 10 μM ifenprodil (d). EPSC frequency (right) was inhibited by 0.1 μM TTX (a) and 30 μM memantine (c, bottom panel). Neither amplitude nor frequency of NMDAR sEPSCs was inhibited by 1 μM memantine (c, top panels) or 10 μM CNQX (e). Inhibitory effects largely reversed during washout. Note that NO-711 is known not to completely washout over a 30 min period[2]. Experimental conditions were identical to those described in the text (Figure 4.2a-e). Values represent mean ± s.e.m. ($n \geq 4$ cells for each condition). *, $P \leq 0.03$ by paired t-test on raw data. Mem: memantine.

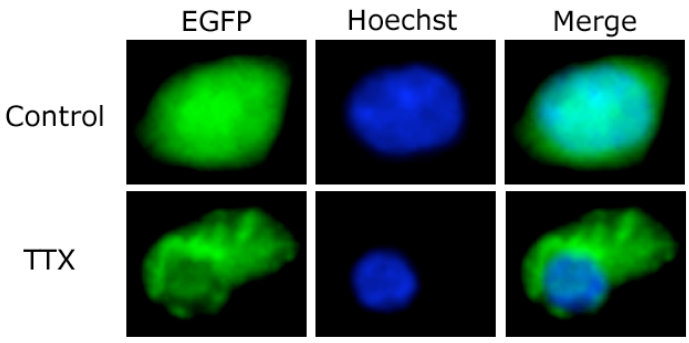


Figure 2.13 Apoptotic changes in mtHtt-transfected neurons after exposure to TTX

Neurons were co-transfected with mtHtt and EGFP, and incubated with TTX. Fluorescent images show transfected neurons (green) and nuclei (blue).

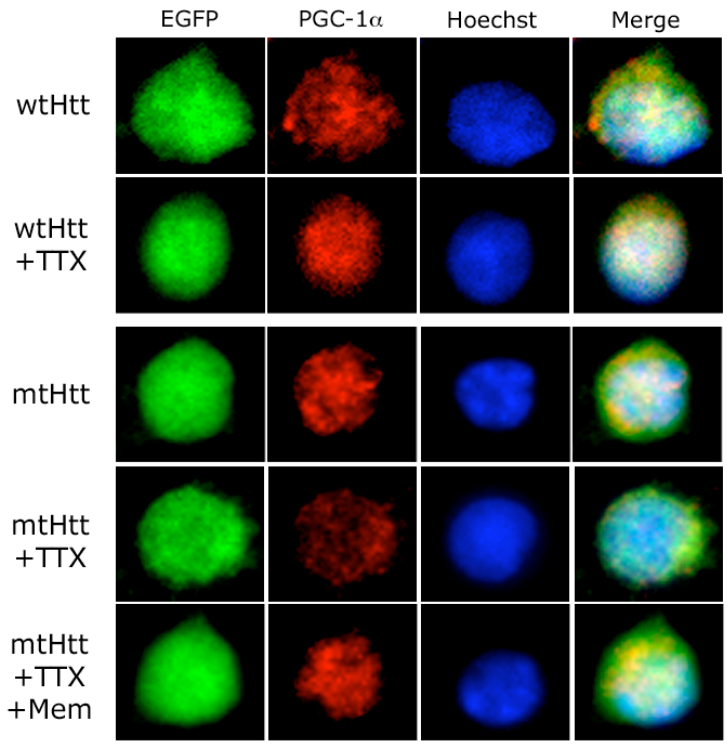


Figure 2.14 Immunofluorescence analysis of PGC-1α levels in Htt-transfected neurons

Neurons were co-transfected with wtHtt or mtHtt plus EGFP and treated with TTX and/or low-dose memantine (10 μM). PGC-1α levels were analyzed by unbaised intensity measurements using SlideBook deconvolution software (Intelligent Imaging Innovations).

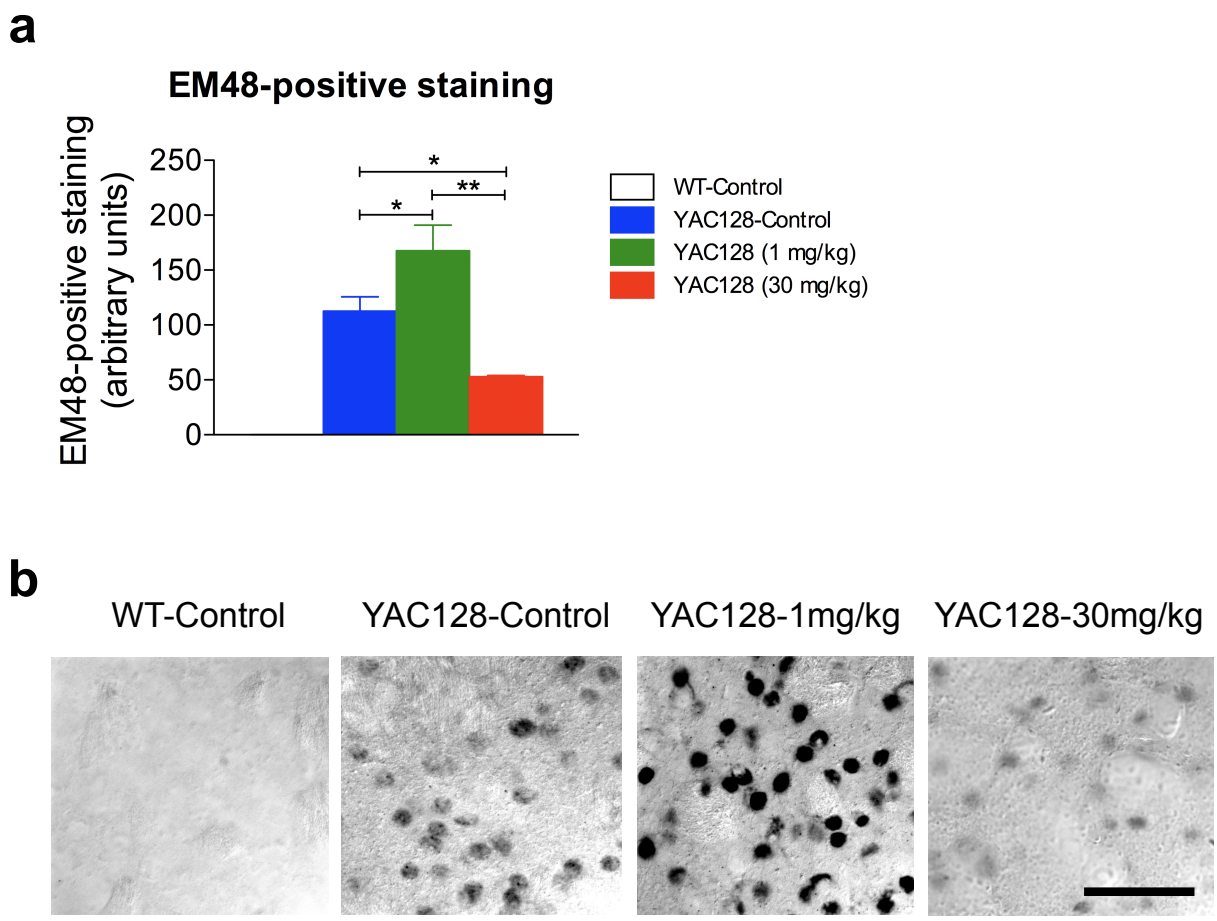


Figure 2.15 Long-term treatment with memantine affects inclusion body formation in a dose-specific manner in transgenic YAC128 HD animals

Animals were treated starting at 2 months of age with 1 mg/kg/day or 30 mg/kg/day of memantine for 10 months, and the extent of inclusion formation was assessed using unbiased densitometry of striatal neurons stained with EM48. No EM48-positive staining was detected in WT animals. EM48-positive staining was significantly lower in YAC128 animals treated with 30 mg/kg compared to untreated YAC128 animals but greater in YAC128 mice treated with 1 mg/kg memantine. (**b**) Representative immunohistological photographs of EM48-stained striata from WT, untreated YAC128 animals, and YAC128 animals treated with 1 or 30 mg/kg memantine. The photomicrographs were taken using an 100x objective (scale bar, 50 mm). Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$.

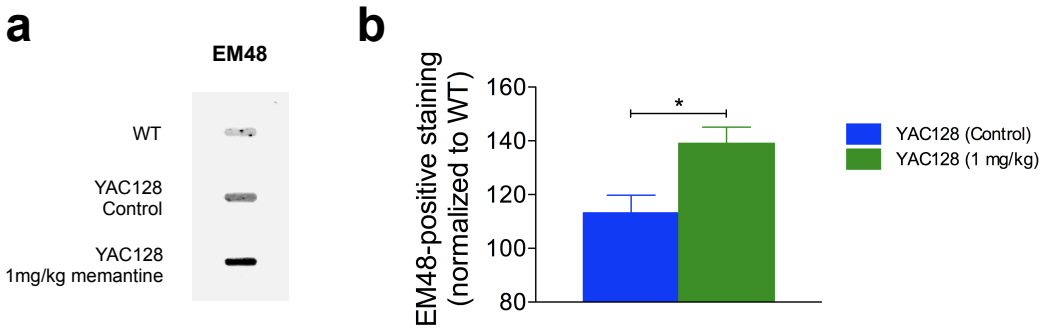


Figure 2.16 Filter trap assay of inclusion in YAC128 HD brains

To validate the increase in inclusion formation observed in YAC128 mice at 12 months of age following treatment with low-dose (1 mg/kg) memantine, a filter trap assay was performed. In this analysis, inclusion formation was assessed in whole-brain lysates using a filter trap assay, and the amount of EM48-positive inclusion was measured. (a) EM48-positive inclusions were significantly higher in YAC128 animals following treatment with 1 mg/kg of memantine compared to untreated YAC128 mice. (b) Densitometry analysis revealed significantly increased EM48-positive inclusion formation in brains of YAC128 animals treated with 1 mg/kg of memantine compared to the untreated group. Values represent mean ± s.e.m.; * $P < 0.05$.

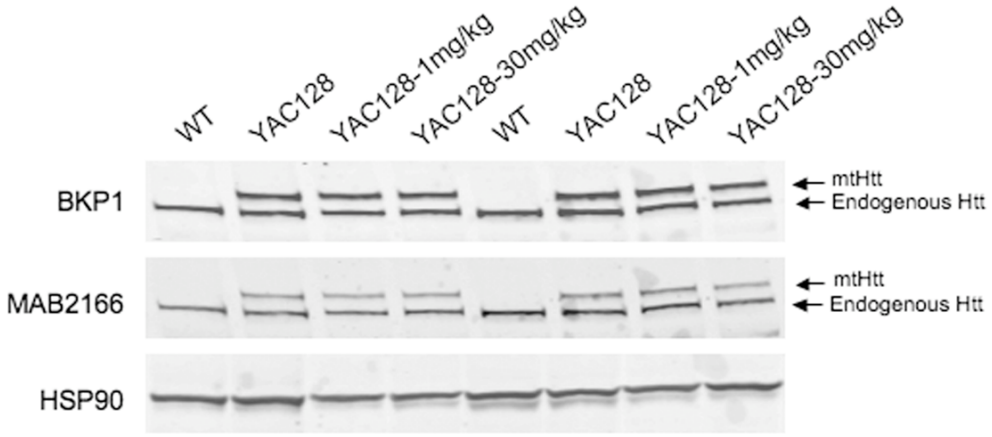


Figure 2.17 Treatment with memantine does not influence endogenous Htt or mtHtt expression levels in YAC128 HD animals

Brains of four month-old mice, including untreated wild-type (WT) and transgenic YAC128 animals along with YAC128 animals treated for two months with 1 mg/kg or 30 mg/kg of memantine, were frozen immediately following euthanasia. Each brain hemisphere was homogenized in 1 ml/100 mg tissue lysis buffer (20 mM Tris/HCl pH 7.2, 10 mM $MgCl_2$, 0.5 mM EDTA, 322 mM sucrose, and 1x complete protease inhibitor cocktail (Roche)) with an automated tissue homogenizer for 30 s. Protein lysates were incubated on ice for 1 h, sonicated for 30 s at 30% output, passaged through a 25G needle. This extraction procedure solubilizes mtHtt from inclusions (Hackam et al., 1998; Kalchman et al., 1997). The brain lysate from each hemisphere (*n* = 4 mice) was loaded onto the same gel. Htt protein was detected using Htt-specific MAB2166 (Chemicon) or polyclonal BKP1 antibody (Kalchman et al., 1997). These antibodies recognize exposed epitopes of Htt, including mtHtt originating in inclusions. HSP90 served as a loading control.

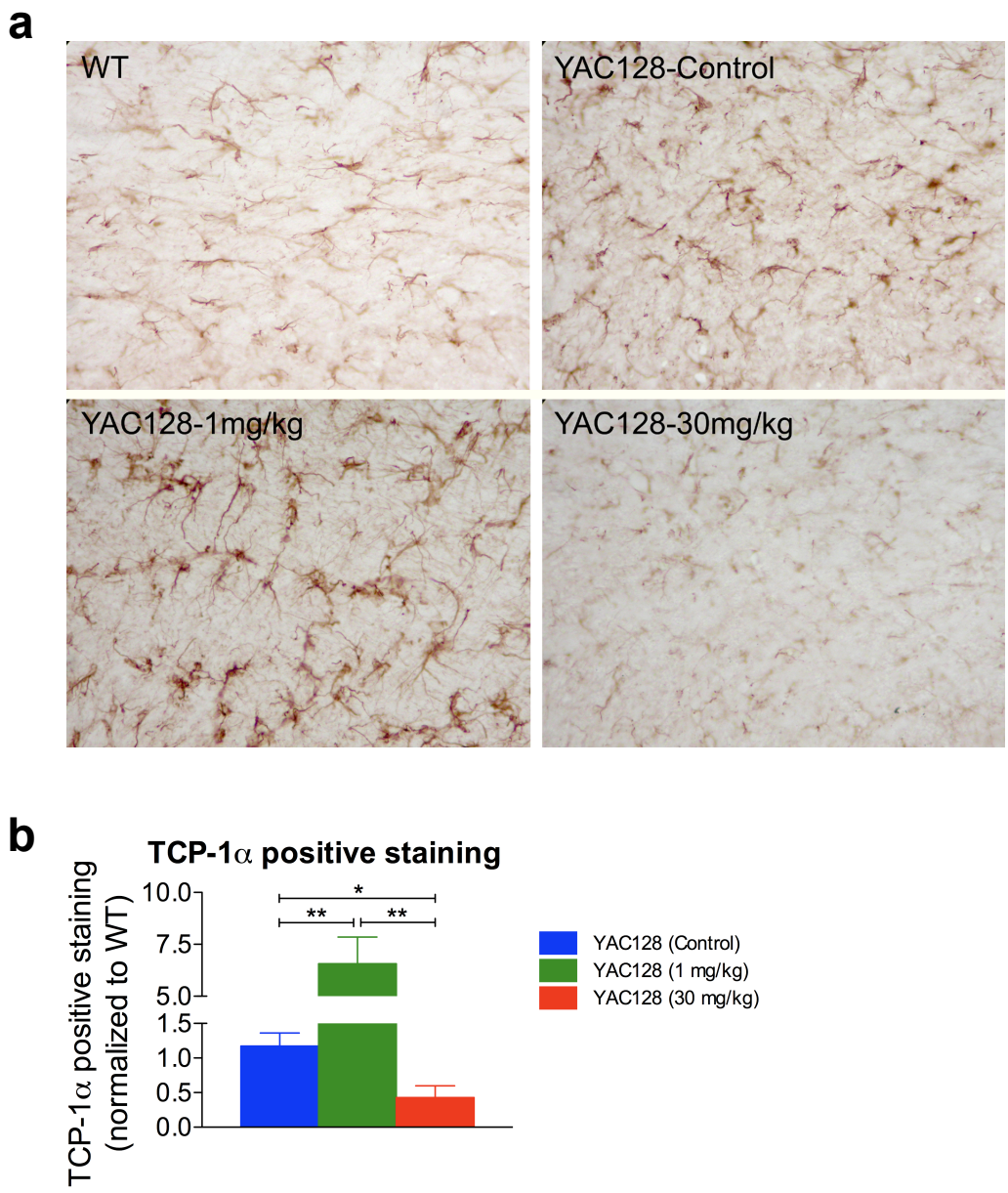


Figure 2.18 Memantine treatment affects TCP-1α expression in a dose-dependent manner in transgenic YAC128 HD animals

Animals were treated starting at 2 months of age with either 1 mg/kg or 30 mg/kg of memantine for 10 months, and expression of TCP-1α was assessed by immunohistochemistry. (a) Positive staining for TCP-1α was detected in both WT and YAC128 animals (40x magnification). (b) Quantification of TCP-1α staining using unbiased percent threshold measurements revealed significantly higher levels in YAC128 animals treated with 1 mg/kg memantine compared to untreated YA128 animals. In contrast, significantly lower levels of TCP-1α staining were detected in animals treated with 30 mg/kg memantine compared to untreated YAC128 animals. There was no significant difference between WT and untreated YAC128 animals. Values represent mean ± s.e.m.; * $P < 0.01$; ** $P < 0.001$.

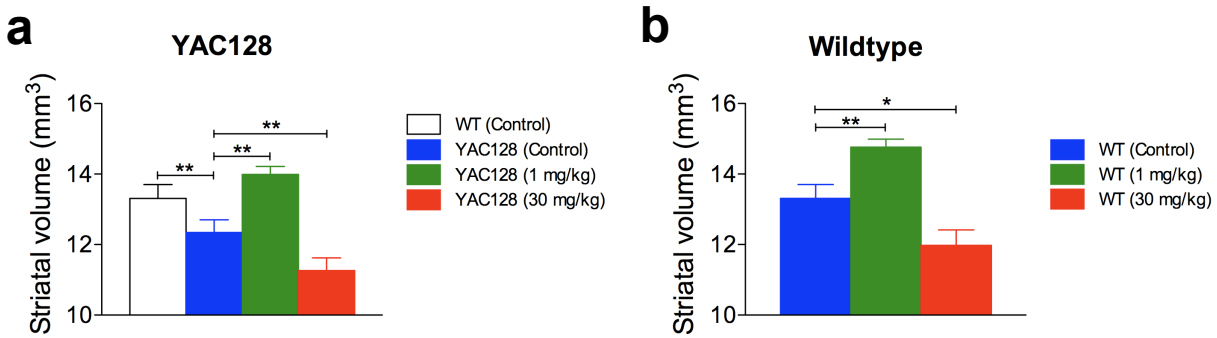


Figure 2.19 Long-term treatment with memantine affects neuropathology in a dose-specific manner in transgenic YAC128 HD animals

Animals were treated starting at 2 months of age with 1 mg/kg/day or 30 mg/kg/day of memantine for 10 months and striatal volume was assessed by stereology. (a) Low-dose memantine treatment in YAC128 animals improved striatal volume compared to untreated YAC128 mice, while high-dose memantine worsened this parameter. (b) Treatment of WT animals with low-dose memantine led to a significant increase in striatal volume compared to untreated WT animals, while high-dose memantine led to a significant decrease in striatal volume compared to untreated WT mice. Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$.

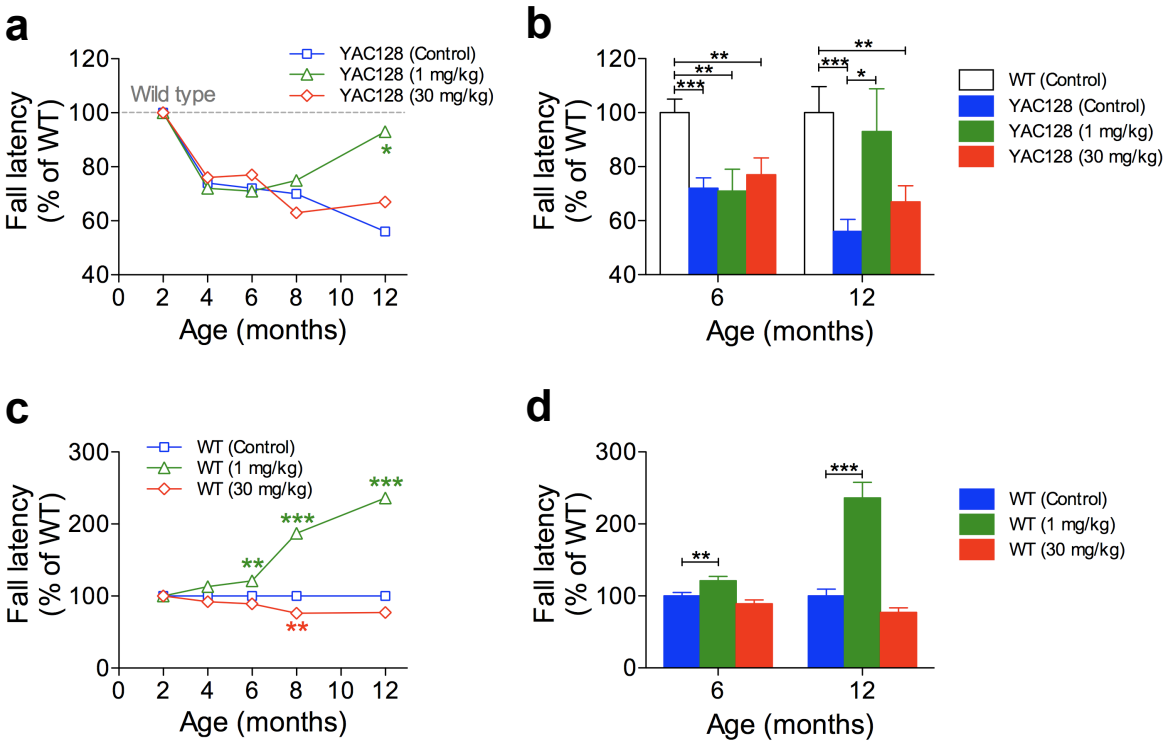


Figure 2.20 Long-term treatment with memantine affects motor function in a dose-specific manner in transgenic YAC128 HD animals

Effects of low-dose or high-dose memantine on the accelerating rotarod task in YAC128 animals. Values represent the mean change from baseline latency to fall. YAC128 animals displayed significantly lower performance compared to WT. YAC128 animals treated with 1 mg/kg (but not 30 mg/kg) memantine manifested a significantly better performance than untreated YAC128 animals. Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

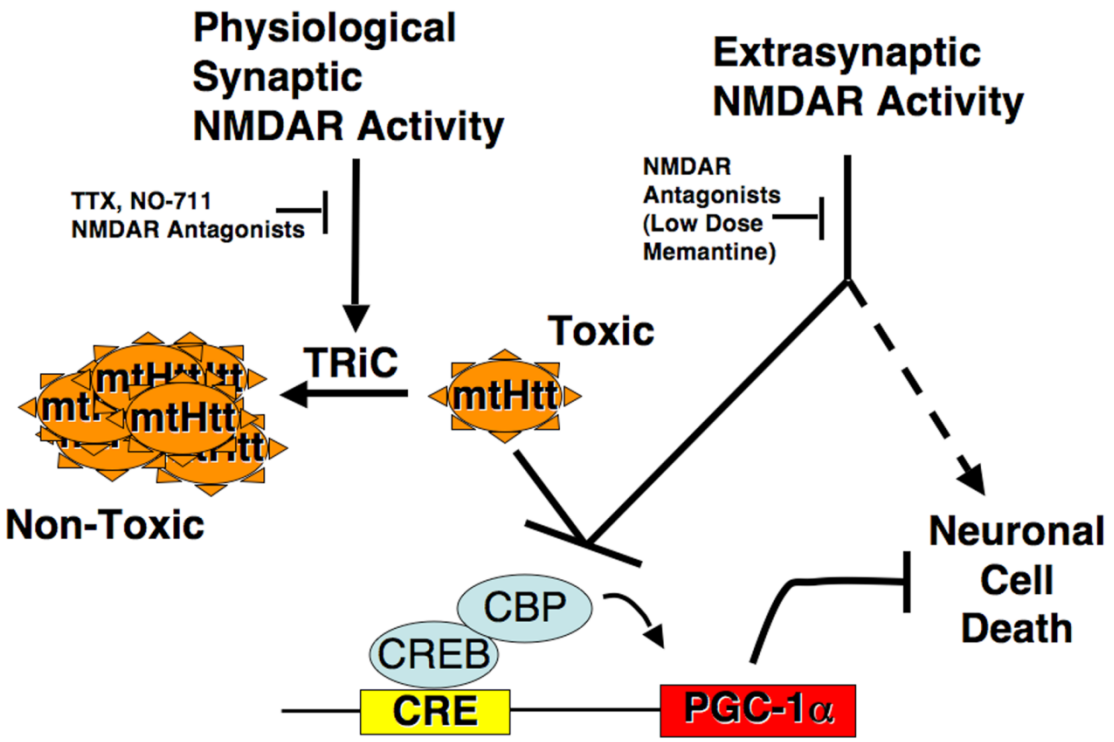


Figure 2.21 Schematic model showing the role of physiological synaptic vs. excessive extrasynaptic NMDAR activity in the neurodegeneration of HD

Physiological synaptic NMDAR activity promotes neuroprotection, in part by facilitating non-toxic aggregation of mtHtt via the chaperonin TRiC. Otherwise toxic mtHtt would interfere with the neuroprotective CREB-PGC-1α pathway. In contrast, extrasynaptic NMDAR activity promotes neuronal cell injury and death. Excessive extrasynaptic NMDAR activity and toxic mtHtt contribute to transcriptional deregulation of the CREB-PGC-1α cascade. Drugs inhibiting extrasynaptic or synaptic activity are indicated. Note that most NMDAR antagonists, as well as high concentrations of memantine, block both synaptic and extrasynaptic NMDAR-mediated currents, while low concentrations of memantine block only the extrasynaptic component, thus relatively sparing synaptic activity and promoting neuroprotection.

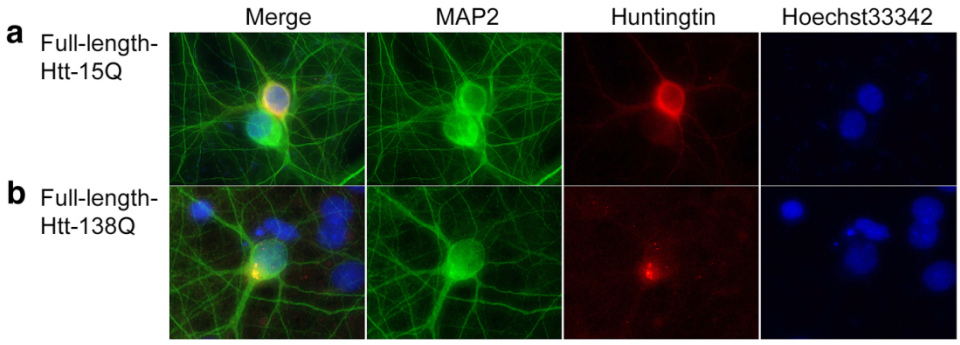


Figure 2.22 Inclusion formation of full-length mtHtt

(a, b) Full-length wtHtt or mtHtt was detected in transfected neurons by immunocytochemistry using anti-huntingtin antibody.

2.4. References

Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R., Finkbeiner, S., 2004. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature. 431, 805-10.

Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J., Martin, J.B., 1986. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 321, 168-71.

Behrends, C., Langer, C.A., Boteva, R., Böttcher, U.M., Stemp, M.J., Schaffar, G., Rao, B.V., Giese, A., Kretzschmar, H., Siegers, K., Hartl, F.U., 2006. Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. Mol Cell. 23, 887-97.

Beister, A., Kraus, P., Kuhn, W., Dose, M., Weindl, A., Gerlach, M., 2004. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. J Neural Transm Suppl. 117-22.

Chen, H.S., Pellegrini, J.W., Aggarwal, S.K., Lei, S.Z., Warach, S., Jensen, F.E., Lipton, S.A., 1992. Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. J Neurosci. 12, 4427-36.

Ciechanover, A., Brundin, P., 2003. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron. 40, 427-446.

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C.N., Tanese, N., Krainc, D., 2006. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 127, 59-69.

Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L., Bates, G.P., 1997. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 90, 537-48.

DiFiglia, M., Sapp, E., Chase, K., Davies, S., Bates, G., Vonsattel, J., Aronin, N., 1997. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science. 277, 1990-3.

Fellin, T., Pascual, O., Gobbo, S., Pozzan, T., Haydon, P.G., Carmignoto, G., 2004. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron. 43, 729-43.

Ferrante, R.J., Andreassen, O.A., Dedeoglu, A., Ferrante, K.L., Jenkins, B.G., Hersch, S.M., Beal, M.F., 2002. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. J Neurosci. 22, 1592-9.

Friedlander, R.M., 2003. Apoptosis and caspases in neurodegenerative diseases. N Engl J Med. 348, 1365-75.

Group, T.H.s.D.C.R., 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, 971-83.

Gutekunst, C.A., Li, S.H., Yi, H., Mulroy, J.S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R.J., Hersch, S.M., Li, X.J., 1999. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci. 19, 2522-34.

Hackam, A.S., Singaraja, R., Wellington, C.L., Metzler, M., McCutcheon, K., Zhang, T., Kalchman, M., Hayden, M.R., 1998. The influence of huntingtin protein size on nuclear localization and cellular toxicity. The Journal of Cell Biology. 141, 1097-105.

Hardingham, G.E., Fukunaga, Y., Bading, H., 2002. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat. Neurosci. 5, 405-14.

Heng, M.Y., Detloff, P.J., Wang, P.L., Tsien, J.Z., Albin, R.L., 2009. In vivo evidence for NMDA receptor-mediated excitotoxicity in a murine genetic model of Huntington disease. J Neurosci. 29, 3200-5.

Hesselink, M.B., De Boer, B.G., Breimer, D.D., Danysz, W., 1999. Brain penetration and in vivo recovery of NMDA receptor antagonists amantadine and memantine: a quantitative microdialysis study. Pharm Res. 16, 637-42.

Kalchman, M.A., Koide, H.B., McCutcheon, K., Graham, R.K., Nichol, K., Nishiyama, K., Kazemi-Esfarjani, P., Lynn, F.C., Wellington, C., Metzler, M., Goldberg, Y.P., Kanazawa, I., Gietz, R.D., Hayden, M.R., 1997. HIP1, a human homologue of S. cerevisiae Sla2p, interacts with membrane-associated huntingtin in the brain. Nat Genet. 16, 44-53.

King, M.A., Goemans, C.G., Hafiz, F., Prehn, J.H.M., Wyttenbach, A., Tolkovsky, A.M., 2008. Cytoplasmic inclusions of Htt exon1 containing an expanded polyglutamine tract suppress execution of apoptosis in sympathetic neurons. J Neurosci. 28, 14401-15.

Kitamura, A., Kubota, H., Pack, C.-G., Matsumoto, G., Hirayama, S., Takahashi, Y., Kimura, H., Kinjo, M., Morimoto, R.I., Nagata, K., 2006. Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. Nat Cell Biol. 8, 1163-70.

Kornhuber, J., Quack, G., 1995. Cerebrospinal fluid and serum concentrations of the N-methyl-D-aspartate (NMDA) receptor antagonist memantine in man. Neuroscience Letters. 195, 137-9.

Lei, S.Z., Pan, Z.H., Aggarwal, S.K., Chen, H.S., Hartman, J., Sucher, N.J., Lipton, S.A., 1992. Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron. 8, 1087-99.

Li, L., Murphy, T.H., Hayden, M.R., Raymond, L.A., 2004. Enhanced striatal NR2B-containing N-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. J Neurophysiol. 92, 2738-46.

Lipton, S., 2007. Pathologically activated therapeutics for neuroprotection. Nat. Rev. Neurosci. 8, 803-808.

Lipton, S.A., Rosenberg, P.A., 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med. 330, 613-22.

Lipton, S.A., 2006. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov. 5, 160-70.

McGill, J.K., Beal, M.F., 2006. PGC-1alpha, a new therapeutic target in Huntington's disease? Cell. 127, 465-8.

Melville, M.W., McClellan, A.J., Meyer, A.S., Darveau, A., Frydman, J., 2003. The Hsp70 and TRiC/CCT chaperone systems cooperate in vivo to assemble the von Hippel-Lindau tumor suppressor complex. Mol Cell Biol. 23, 3141-51.

Milnerwood, A.J., Gladding, C.M., Pouladi, M.A., Kaufman, A.M., Hines, R.M., Boyd, J.D., Ko, R.W., Vasuta, O.C., Graham, R.K., Hayden, M.R., Murphy, T.H., Raymond, L.A., 2010. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron. 65, 178-90.

Minkeviciene, R., Banerjee, P., Tanila, H., 2004. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. J Pharmacol Exp Ther. 311, 677-82.

Mouginot, D., Kombian, S., Pittman, Q., 1998. Activation of presynaptic GABAB receptors inhibits evoked IPSCs in rat magnocellular neurons in vitro. J. Neurophysiol. 79, 1508-1517.

Nucifora , F.C., Sasaki, M., Peters, M.F., Huang, H., Cooper, J.K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V.L., Dawson, T.M., Ross, C.A., 2001. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science. 291, 2423-8.

Okamoto, S.-i., Li, Z., Ju, C., Scholzke, M.N., Mathews, E., Cui, J., Salvesen, G.S., Bossy-Wetzel, E., Lipton, S.A., 2002. Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. Proc Natl Acad Sci USA. 99, 3974-9.

Parsons, C.G., Stöffler, A., Danysz, W., 2007. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. Neuropharmacology. 53, 699-723.

Pickett, J., 2006. Folding away the bad guys. Nature Reviews Molecular Cell Biology.

Sánchez, I., Mahlke, C., Yuan, J., 2003. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. Nature. 421, 373-9.

Saudou, F., Finkbeiner, S., Devys, D., Greenberg, M.E., 1998. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell. 95, 55-66.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12, 1555-67.

Tam, S., Geller, R., Spiess, C., Frydman, J., 2006. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol. 8, 1155-62.

Tian, G.-F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., Oberheim, N., Lou, N., Wang, X., Zielke, H.R., Kang, J., Nedergaard, M., 2005. An astrocytic basis of epilepsy. Nat Med. 11, 973-81.

Tovar, K.R., Westbrook, G.L., 1999. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci. 19, 4180-8.

Tsai, J., Grutzendler, J., Duff, K., Gan, W.-B., 2004. Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. Nat. Neurosci. 7, 1181-3.

Van Raamsdonk, J.M., Pearson, J., Slow, E.J., Hossain, S.M., Leavitt, B.R., Hayden, M.R., 2005. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci. 25, 4169-80.

Vonsattel, J.P., Myers, R.H., Stevens, T.J., Ferrante, R.J., Bird, E.D., Richardson, E.P., 1985. Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol. 44, 559-77.

Zeron, M.M., Hansson, O., Chen, N., Wellington, C.L., Leavitt, B.R., Brundin, P., Hayden, M.R., Raymond, L.A., 2002. Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron. 33, 849-60.

3. Rasagiline treatment improves motor function in the YAC128 mouse model of Huntington disease[2]

3.1. Introduction

Huntington Disease (HD) is an autosomal dominant disease characterized by motor, psychiatric, and cognitive deficits that are relentlessly progressive in nature and ultimately fatal, resulting in death typically 10-15 years following onset (Hayden, 1981). The disease is caused by a CAG trinucleotide expansion in exon 1 of the *HD* gene, resulting in an expanded polyglutamine tract in the gene product, huntingtin (htt) (Group, 1993). Despite expression of htt throughout the brain, the neuronal loss observed in HD is selective, primarily affecting medium spiny neurons (MSNs) within the striatum (Kremer et al., 1992).

Considerable progress in our understanding of the pathogenesis of HD has been made and a number of mechanisms contributing to the neuronal loss have been identified, including transcriptional dysregulation, oxidative stress, energy metabolism deficits, and htt proteolysis (Graham et al., 2006; Roze et al., 2008). Ample evidence also supports a role for excitotoxicity, a process in which excessive glutamate signaling, particularly via the NMDA-type glutamate receptors (NMDAR), leads to disrupted intracellular calcium homeostasis and cell death, in the neuronal loss in HD. Mutant htt-mediated disruption of mitochondrial calcium handling is thought to play a key role in this process (Pouladi et al., 2006).

Indeed, defects in mitochondrial calcium handling have been identified in both HD patients and mouse models of HD. For example, mitochondria from lymphoblasts of HD patients have a

[2] A version of this chapter is in preparation for submission. Pouladi MA, Dar Santos R, Bertram LN, Zapala M, Lu G, Leavitt BR, Hayden MR. Rasagiline treatment improves motor function in the YAC128 mouse model of Huntington disease.

compromised calcium buffering capacity and are less resistant to induction of the mitochondrial permeability transition (mPT) upon calcium challenge compared to normal individuals (Panov et al., 2002). Similarly, mitochondria from brains of the transgenic YAC72 mice expressing human mutant htt with a CAG repeat length of 72 are less resistant to calcium challenge than mitochondria from brain of wildtype (WT) animals or transgenic YAC18 control animals expressing human huntingtin with a CAG repeat length of 18 (Panov et al., 2002). This has the effect of activating the mPT and causing the release of calcium and apoptotic factors at sub-threshold levels, effectively facilitating calcium dysregulation and the induction of cell death. Furthermore, using primary medium spiny neurons (MSN) from transgenic YAC46 mice expressing human mutant huntingtin with a polyglutamine tract length of 46, it was demonstrated that inhibition of the mPT with cyclosporine A, bongkrekic acid, or boosting mitochondrial function with coenzyme Q10, substantially diminishes NMDAR-mediated excitotoxicity (Zeron et al., 2004) and abolishes the observed difference in NMDAR-mediated excitotoxic cell death between YAC46 and WT. Finally, studies in MSNs from YAC128 animals expressing human mutant Htt with a polyglutamine tract length of 128 showed enhanced induction of the mPT and excitotoxic cell death following NMDAR-mediated calcium influx, an effect that was prevented by inhibitors of the mPT (Fernandes et al., 2007; Tang et al., 2005).

Despite these advances in understanding the pathogenic mechanisms involved in the disease process, no clinically-proven treatment for HD presently exists. Given the available evidence pointing to a role for aberrant mitochondrial calcium handling in HD, we explore in this study the therapeutic potential of rasagiline, a propargylamine shown to prevent the induction of the mPT, as a strategy to modulate and mitigate excitotoxicity for the treatment of HD.

Rasagiline is a selective irreversible inhibitor of monoamine oxidase type B (MAO-B) (Finberg et al., 1981) and has been shown to have benefits in Parkinson's disease (PD) both as a monotherapy in early disease (Group, 2002) and as an adjunct therapy in moderate to advanced disease (Group, 2005; Rascol et al., 2005). The potential efficacy of rasagiline in protecting against excitotoxicity was initially suggested by studies showing that treatment with deprenyl (selegiline), a propargyl structurally related to rasagiline, protected dopaminergic neurons against NMDAR-mediated excitotoxicity (Mytilineou et al., 1997). Rasagiline has since been shown to be neuroprotective in the middle cerebral artery occlusion (MCAO) and stroke-prone spontaneously hypertensive rodent models of stroke where excitotoxicity is thought to play a prominent role (Eliash et al., 2001; Speiser et al., 1999; Speiser et al., 2007). Treatment with rasagiline has also been shown to protect against various neurotoxic insults in a number of cellular and animal studies (Eliash et al., 2001; Eliash et al., 2009; Finberg et al., 1998; Kupsch et al., 2001; Maruyama et al., 2001; Speiser et al., 1998; Speiser et al., 1999; Speiser et al., 2007; Stefanova et al., 2008). Significantly, the extent of neuroprotection afforded by rasagiline is similar to that of TV-1022, its optical isomer that is 1000 times less potent as an MAO inhibitor, indicating that rasagiline's neuroprotective properties are largely independent of its MAO inhibitory activity.

Structure activity studies have shown that the neuroprotective properties of rasagiline are attributed to its propargyl moiety, which mediates anti-apoptotic induction of Bcl-2 and PKC and down-regulation of the pro-apoptotic proteins FAS and Bax (reviewed in (Youdim et al., 2005)). Rasagiline has also been shown to attenuate oxidative stress-related cellular damage by up-regulating the anti-oxidative enzymes superoxide dismutase and catalase (Maruyama et al., 2000). Importantly, these effects of rasagiline have been shown to result in stabilization of the

mitochondrial membrane potential (Maruyama et al., 2001) and prevention of the induction of the mPT and the subsequent apoptotic cascade leading to cellular demise (Akao et al., 2002; Mandel et al., 2005; Maruyama et al., 2000; Maruyama et al., 2001), which likely underlie its protective effects in cellular and animal models of neurotoxicity.

Based on the ability of rasagiline to attenuate oxidative stress-mediated cellular damage, stabilize the mitochondrial membrane potential, and prevent the induction of the mPT, we investigated the efficacy of rasagiline in the transgenic YAC128 mouse model of HD. The YAC transgenic mice express the entire human HD gene under the control of the endogenous huntingtin promoter and regulatory elements and recapitulate many behavioural and neuropathological features of the human condition, making them particularly suited for evaluation of therapeutic interventions (Slow et al., 2003; Van Raamsdonk et al., 2005b). These features include motor dysfunction starting at 3 months of age and selective striatal neuronal deficits at 9 months of age. We demonstrate that treatment with rasagiline attenuates the effects of oxidative stress on viability of MSNs ex vivo, and reduces excitotoxicity-related striatal lesioning in vivo. We further demonstrate that long-term treatment with rasagiline improves motor function in the transgenic YAC128 HD animals.

3.2. Materials and methods

Animals

Male and female YAC128 mice expressing expanded human huntingtin with 128 CAG repeats and WT littermates maintained on the FVB/N strain (Charles River, Wilmington, MA) were used for these experiments (Slow et al., 2003). Mice were housed singly or in pairs in duplex cages with littermates of mixed genotype and maintained under a 12 L:12 D light cycle (lights on at 2300) in a clean facility and given free access to food and water. Experimenters were blind to the genotype of the mice. All experiments were performed with the approval of the animal care committee at the University of British Columbia.

Quinolinic Acid and Malonate Lesions

Three month old FVB mice were pretreated with PBS or rasagiline (5 mg/kg). Mice were anesthetized with avertin (2.5%) and placed in a stereotaxic instrument. To induce striatal lesions, animals were injected intrastriatally with quinolinic acid (QA; 20 nmol) or malonate (0.6 μM) 30-min post-rasagiline treatment. Coordinates for the unilateral striatal injections were as follows: 0.8 mm posterior to Bregma, 1.8 mm lateral from the midline, and 3.5 mm below the dorsal surface of the neocortex.

Seven days after the intrastriatal injection, animals were injected with heparin and terminally anesthetized with intraperitoneally-injected 2.5% avertin. The brains were extracted and treated as described in the brain sample preparation section. For each mouse, coronal 25 μm sections spaced 200 μm apart throughout the striatum were stained with FluoroJade B (Histo-Chem, Jefferson, Arkansas), a fluorescent stain that labels degenerating neurons in fixed brain sections,

and the total striatal lesion volume was determined using unbiased techniques and StereoInvestigator software (Microbrightfield, Williston, VT).

H_2O_2 Neurotoxicity

Embryos were isolated from E15.5 FVB/N time-pregnant females. Brain tissue was dissected and striatal neuronal cultures were set up as described previously (Metzler et al., 2007).

To determine the effect of rasagiline on H_2O_2-mediated neurotoxicity, striatal neurons were differentiated for 14 days in vitro. Neurons were then pre-incubated with 10mM rasagiline for 20 min, or with an equal volume of PBS followed by a 3-hr incubation with 10uM of H_2O_2. Subsequently neurons were washed in ice-cold PBS and neuronal viability was assessed using Promega CellTiter-Glo ATP Assay (Promega).

Drug treatment

Rasagiline mesylate was provided by Teva Pharmaceutical Inc, and all reported doses refer to the mesylate salt. Two doses of 0.1 mg/kg and 1 mg/kg, administered in the drinking water, were chosen for the trial based on the recommendations of Teva Pharmaceuticals Inc (Dr. Eran Blaugrund, Teva Pharmaceuticals Inc, personal communication). For administration in the drinking water, water consumption of individual cages was monitored on a biweekly basis along with animal body weights. The concentrations of drug solutions for each cage were then adjusted accordingly. The drug solutions were replaced twice/week and were provided ad libitum.

Motor function assessment

Training and baseline testing for motor function tasks was carried out at 2 months of age. Testing took place during the dark cycle and was curried out every two months between 2 and 12 months of age. Motor co-ordination and balance was assessed using accelerating and fixed rotarod tasks (UGO Basile, Comerio, Italy). In the accelerating task, the rotarod accelerated from 5 revolutions

per minute (RPM) to 40 RPM over 5 minutes. In the fixed task, the rotarod revolved at 24 RPM. Performance in the rotarod tasks was assessed by the amount of time that a mouse could remain running on the rotarod; the maximum scores in the accelerating and fixed tasks are 300 seconds and 60 seconds, respectively. During training, mice were given three trials per day for three consecutive days. Rotarod scores are the average of three trials spaced 2 h apart.

Brain sample preparation

Mice were injected with heparin followed by terminal anesthesia with intraperitoneally-injected 2.5% avertin. The animals were perfused with 3% paraformaldehyde with 0.6% glutaraldehyde in phosphate-buffered saline (PBS). Brains were left in the skulls for 24 hrs in 3% paraformaldehyde, then removed and stored in PBS. After weighing, the brains were transferred to a 30% sucrose solution containing 0.08% sodium azide in PBS. They were then frozen on dry ice, mounted with Tissue-TEK O.C.T. compound (Sakura, Torrance, CA), and sliced coronally into 25 μm sections on a cryostat (Microm HM 500M, Richard-Allan Scientific, Calamazoo, MI). The sections are collected and stored in PBS with 0.08% sodium azide at 4 degrees.

Neuropathological analysis

A series of 25 μm-thick coronal sections spaced 200 μm apart spanning the striatum were stained with NeuN antibody (1:100; Chemicon, Temecula, CA) overnight at room temperature, followed by incubation with biotinylated anti-mouse antibody (1:200; Vector Laboratories, Burlingame, CA). The signal was amplified with an ABC Elite kit (Vector) and detected with diaminobenzidine (DAB; Pierce, Rockford, IL). Striatal volume was determined from a series of mounted sections using StereoInvestigator software (Microbrightfield, Williston, VT). Briefly, striatal volumes were determined by tracing the perimeter of the striatum in serial sections spanning the striatum.

Statistical analysis

Data are expressed as means±SEM. Whenever suitable, results were interpreted using one-way ANOVA with a Student-Newman-Keuls (SNK) post-hoc test. Pairwise comparisons between genotypes/treatments at individual time points were assessed with a Student's t-test. Differences were considered statistically significant when $P < 0.05$.

3.3. Results

Treatment with rasagiline protects against H_2O_2-mediated neurotoxicity in primary striatal neurons

Oxidative stress has been shown to play a role in excitotoxicity and neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD), and HD. To evaluate the neuroprotective potential of rasagiline against oxidative stress-mediated neurotoxicity, primary striatal neurons were pre-treated with rasagiline for 20 min followed by a 3-hr incubation with 10μM H_2O_2. Treatment with H_2O_2 results in a significant decrease in viability, an effect that was prevented by pre-treatment with rasagiline (Figure 3.1a).

Rasagiline treatment protects against and malonate- and quinolinic acid-mediated excitotoxicity in vivo

To examine whether treatment with rasagiline can protect against excitotoxicity-mediated striatal lesioning, WT animals were pre-treated with rasagiline (5 mg/kg, i.p.) or an equal volume of PBS and injected intrastriatally 30 min later with the mitochondrial toxin malonate, the NMDA receptor agonist QA, or PBS. Both malonate and QA have been shown to induce excitotoxicity-mediated lesioning when injected into the striatum. Treatment with rasagiline protected against both malonate- and QA-mediated excitotoxicity, as signified by decrease lesion volume compared to PBS-treated animals (Figure 3.1b, c).

Long-term treatment with rasagiline leads to improved motor function in the YAC128 HD animals

Transgenic YAC128 HD animals display progressive motor deficits signified by reduced time on the rotating rod in the accelerating rotarod test of motor coordination compared to WT animals (Slow et al., 2003). To assess the impact of rasagiline on motor function, animals were treated with 0.1 mg/kg or 1 mg/kg of rasagiline starting at 2 months of age and their motor function as

assessed by performance on the rotarod task was evaluated at 2, 4, 6, 8, and 12 months of age. Untreated YAC128 animals showed significantly reduced performance at 4, 6, 8, and 12 months of age compared to WT animals (Figure 3.2a; $P < 0.05$ for 4 months of age, and $P < 0.001$ for 6, 8, and 12 months of age). YAC128 animals treated with 0.1 mg/kg of rasagiline had a significantly improved performance at 4 months of age compared to WT ($P < 0.05$) but were not statistically different than WT at 6, 8, and 12 months of age (Figure 3.2a). Furthermore, YAC128 animals treated with 0.1 mg/kg rasagiline had a significantly improved performance compared to untreated YAC128 animals at 4 ($P < 0.001$), 6 ($P < 0.01$), 8 ($P < 0.01$), and 12 ($P < 0.05$) months of age (~1.5×, 2×, 2×, and 1.5× improvement over untreated at 4, 6, 8, and 12 months of age, respectively) (Figure 3.2a). YAC128 animals treated with 1 mg/kg had a significantly lower performance at 4, 6, 8, and 12 months compared to WT ($P < 0.01$ for 4 months of age, and $P < 0.001$ for 6, 8, and 12 months of age).

The impact of rasagiline on motor function in WT animals was also evaluated. The performance of WT animals treated with 0.1 mg/kg was not statistically different than that of untreated WT animals at 8 months of age, but was significantly improved at 4, 6, and 12 months of age compared to untreated WT (Figure 3.2c; $P < 0.05$ for 4 and 6 months of age, and $P < 0.01$ for 12 months of age). The performance of WT animals treated with 1 mg/kg rasagiline was not statistically different than that of untreated WT animals at any of the time points measured (Figure 3.2c).

Long-term treatment with rasagiline fails to rescue striatal neuropathology in the YAC128 HD animals

The effect of long-term rasagiline treatment on neuropathology in the YAC128 HD animals was assessed. The brain weights of untreated and rasagiline-treated YAC128 animals were not statistically different (Figure 3.3a). The striatal volumes and neuronal counts of YAC128 animals

treated with 0.1 mg/kg were significantly decreased compared to untreated YAC128 animals, while the striatal volume and neuronal counts of YAC128 animals treated with 1 mg/kg rasagiline were not different compared to untreated YAC128 animals (Figure 3.3c, d).

The brain weights and striatal volumes of rasagiline-treated WT animals were significantly increased compared to untreated WT animals (Figure 3.4a, b). Furthermore, the neuronal counts of WT animals treated with 1 mg/kg, but not 0.1 mg/kg, were significantly increased compared to untreated WT animals (Figure 3.4c).

3.4. Discussion

In this study we demonstrate that rasagiline treatment reduces oxidative stress-mediated cell death and excitotoxicity in striatal neurons. We demonstrate that treatment with rasagiline in transgenic YAC128 HD animals leads to improved performance in the rotarod test of motor function, both early and late in the disease phenotype.

Treatment of primary MSNs with rasagiline attenuated the decrease in viability mediated by H_2O_2 (Figure 3.1a). H_2O_2 challenge has been shown to induce oxidative stress-related neurotoxicity via an apoptotic pathway that involves acute elevation in intracellular calcium levels (Hoyt et al., 1997; Whittemore et al., 1995). That rasagiline protected against H_2O_2-mediated toxicity is consistent with its ability to up-regulate anti-oxidative stress enzymes such as superoxide dismutase and catalase, as well as its ability to stabilize the mitochondrial membrane potential, improving the mitochondrial capacity to handle calcium loads and preventing the induction of the mPT (Maruyama et al., 2000). Similar protection by rasagiline is seen following treatment with the peroxynitrite-generating compound *N*-morpholino sydonimine (SIN-1) in the human dopaminergic neuroblastoma cells SH-SY5Y (Maruyama et al., 2000).

Excitotoxicity which leads to disrupted intracellular calcium homeostasis and ultimately results in cell death, has long been implicated in the pathogenesis of HD. Rasagiline treatment protected against malonate-induced striatal lesioning in vivo (Figure 3.1b). Malonate, a reversible inhibitor of succinate dehydrogenase (complex II), has been shown to cause excitotoxic striatal lesions that closely resemble the histological and neurochemical features of HD (Beal et al., 1993). The process by which malonate is thought to act involves inhibition of oxidative phosphorylation and decreased cellular ATP levels leading to partial membrane depolarization and subsequent

NMDAR activation and excitotoxic cell death (Beal et al., 1993; Henshaw et al., 1994; Schulz et al., 1997). Treatment with rasagiline was also protective in the QA model of excitotoxicity in vivo (Figure 3.2b), where activation of NMDAR is achieved by direct injection of its agonist QA into the striatum, resulting in excitotoxic lesioning with neurochemical features that closely resemble those observed in HD (Beal et al., 1986). These data suggest that rasagiline-mediated attenuation of NMDAR-induced excitotoxicity could contribute to its efficacy in treating the behavioural and neuropathological deficits in animal models of HD and potentially human HD.

A case study by Patel and colleagues reporting on the treatment of a 19-year old HD patient in which deprenyl (selegiline), a compound that shares the putative neuroprotective propargyl moiety with rasagiline, in conjunction with fluoxetine led to significant behavioral and motor improvements with no adverse effect is supportive that use of this class of compounds may be beneficial in HD (Patel et al., 1996). In our study, rasagiline treatment resulted in dramatic improvements in motor function of the YAC128 HD mice as assessed by the rotarod test that were observed at the earliest time points assessed and that were sustained throughout the trial (Figure 3.2a,b). It has been proposed that striatal dopaminergic signaling serves to augment the NMDAR-mediated excitotoxicity in HD (Tang et al., 2007). The improvements were dose-specific, with 0.1 mg/kg but not 1 mg/kg leading to improvements, which may reflect the increased MAO-B inhibitory activity in the 1 mg/kg treatment group, with the beneficial effects of the propargyl moiety of rasagiline being offset by the expected increases in dopamine levels and activity. Importantly, no worsening of motor function was observed in the 1 mg/kg treatment group. Further, this observation of dose-specific effects underlies the importance of dosage and dose titration regimens for achieving maximal benefits while minimizing any potential side effects. Furthermore, similar improvements in motor function following treatment with 0.1

mg/kg were observed in WT animals. That improvements in motor function are also observed in WT animals suggests that treatment with rasagiline confers benefits that are not necessarily specific to the disease process in HD (Figure 3.2c,d).

Assessment of neuropathology following treatment with rasagiline showed no improvements in any of the parameters considered in the YAC128 HD mice (Figure 3.3). In a study employing a non-human primate MPTP model of PD, rasagiline treatment was found to decrease of the cell sizes of nigral tyrosine hydroxylase positive neurons, despite significant histological and behavioural improvements in the rasagiline-treated compared to the untreated group (Kupsch et al., 2001). While the cause of such shrinkage in neuronal size reported is not clear, this decrease in cell size may underlie the absence of rescue of striatal deficits following treatment in the YAC128 animals, although that no such decrease in striatal volume is observed in WT animals following treatment (Figure 3.4) argues against this being a generalized effect of rasagiline. Furthermore, that dramatic improvement in motor function is observed in the absence of rescue of striatal deficits in the YAC128 HD mice suggests that different disease processes may underlie the motor dysfunction and the neuronal loss observed, or that the motor function improvements reflect improved mitochondrial health and function following treatment. Similar disconnect has previously been reported for the compound ethyl-EPA, where treatment resulted in improvements of motor function but no rescue of neurodegeneration in the YAC128 HD mice (Van Raamsdonk et al., 2005a).

Although an ideal therapy for HD would ameliorate both the motor dysfunction and rescue the striatal deficits, treatments such as rasagiline that prevent the motor dysfunction or significantly delay its onset would still be beneficial. This is particularly relevant in light of the dearth of effective and clinically-available treatments for HD. Motor dysfunction, or chorea, in HD

patients represents a major disability that severely limits the ability of patients work and perform daily functions. Treatments that attenuate motor dysfunction are likely to lead to dramatic improvements in quality of life and to be of great benefit to HD patients.

In summary, we demonstrate treatment in rasagiline attenuates oxidative stress- and excitotoxicity-mediated neuronal death ex vivo and in vivo. We further demonstrate that treatment with rasagiline improves the motor dysfunction but does not rescue the neurodegeneration in the YAC128 mouse model of HD.

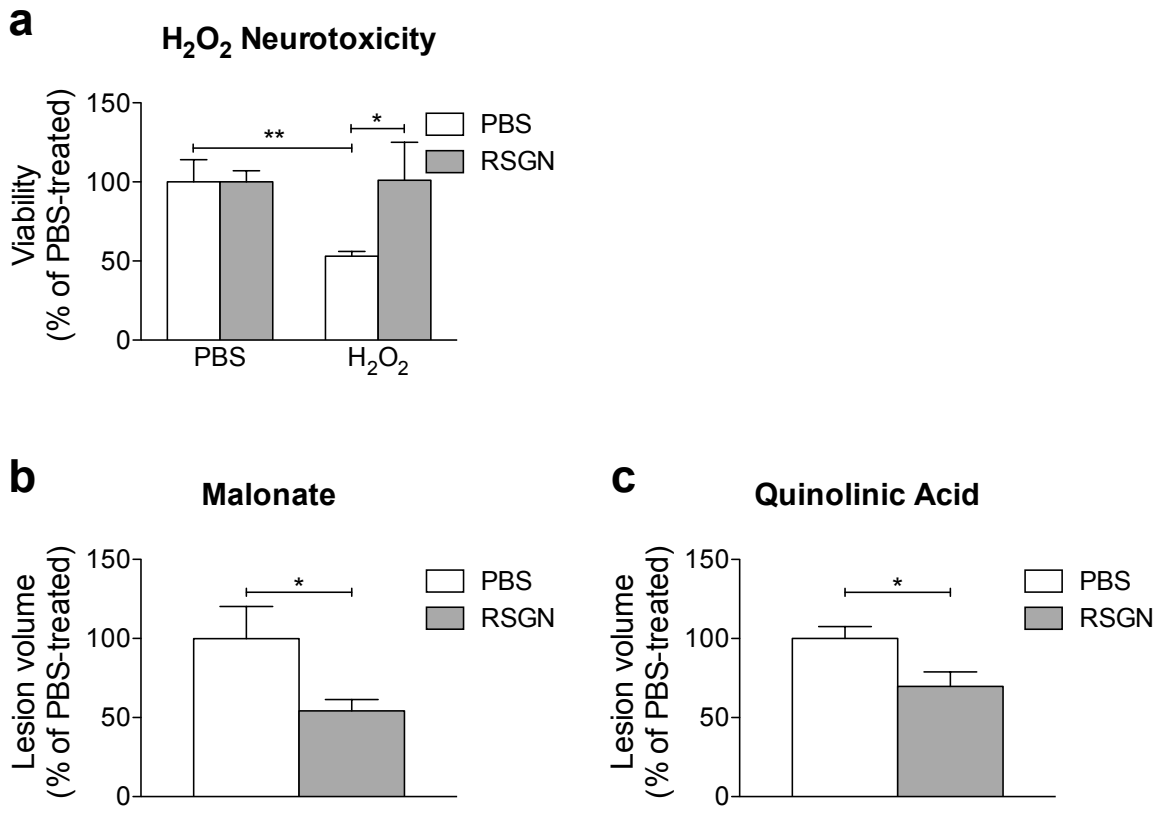


Figure 3.1 Treatment with rasagiline protects striatal neurons against neurotoxins in vitro and in vivo.

To assess the effects of rasagiline on H_2O_2-mediated neurotoxicity, primary striatal neurons were pre-incubated with 10mM rasagiline or with an equal volume of PBS for 20 min, followed by a 3-hr incubation with 10uM of H_2O_2. Neuronal viability following treatment was assessed using Promega CellTiter-Glo ATP Assay. Pre-treatment with rasagiline resulted in decrease neuronal death (increased viability) following H_2O_2 treatment (a). To examine whether rasagiline can protect against excitotoxic neuronal death, FVB/N animals were pre-treated with 5 mg/kg of rasagiline followed by intrastriatal injection of malonate (b) or QA (c). Treatment with rasagiline resulted in significantly decrease striatal lesion volume following both malonate and QA treatment (b, c). Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$.

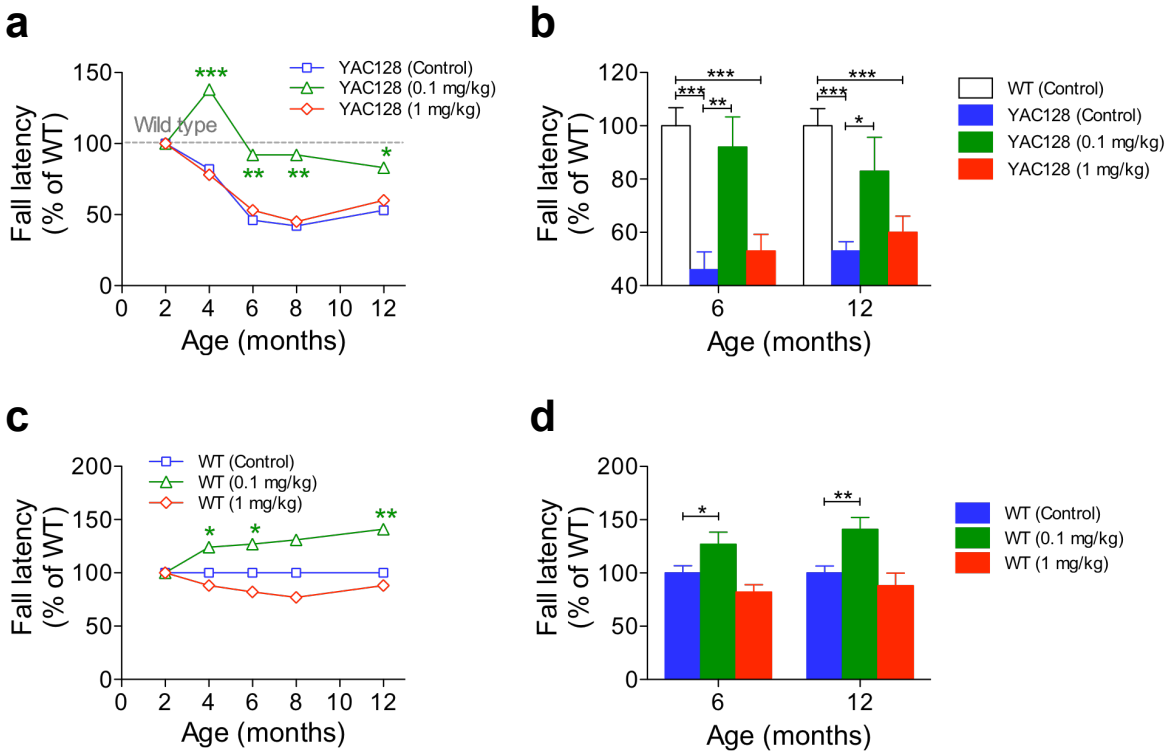


Figure 3.2 Treatment with rasagiline affects motor function in a dose-specific manner in transgenic YAC128 HD animals.

Effects of low-dose or high-dose rasagiline on the accelerating rotarod task in YAC128 (a, b) and WT (c, d) animals. Values represent the mean change from baseline latency to fall. YAC128 animals displayed significantly lower performance compared to WT. YAC128 animals treated with 0.1 mg/kg (but not 1 mg/kg) rasagiline manifested a significantly better performance than untreated YAC128 animals. Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

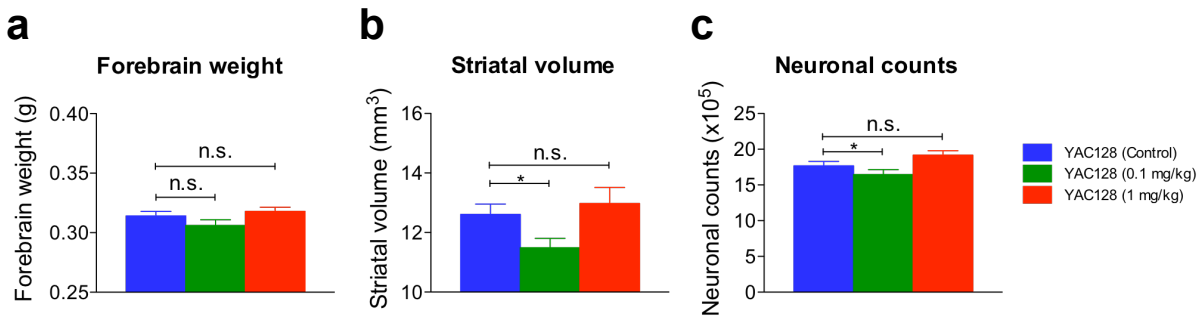


Figure 3.3 Long-term treatment of YAC128 HD animals with rasagiline fails to rescue striatal pathology.

To determine the effect of rasagiline treatment on neuropathology in YAC128 HD animals, 2 months old YAC128 animals were treated with 0.1 or 1 mg/kg rasagiline for 10 months and brain weight, striatal volume, and neuronal counts were assessed at 12 months of age. Treatment of YAC128 animals with 0.1 mg/kg or 1 mg/kg rasagiline failed to improve brain weight (a), striatal volume (b) or striatal neuronal counts (c). Data represent mean ± s.e.m. *, $P < 0.05$; n.s.=no statistical significance.

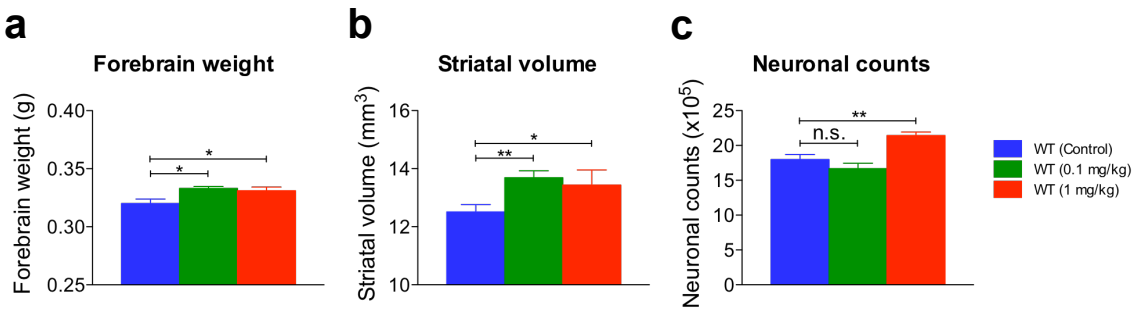


Figure 3.4 Long-term treatment of WT animals with rasagiline increases brain weight, striatal volume and neuronal counts.

Effects of rasagiline treatment on brain weight, striatal volume, and striatal neuronal counts were examined in WT animals. Treatment of WT animals with 0.1 mg/kg or 1 mg/kg rasagiline significantly increase brain weight (a) and striatal volume (b). Furthermore, treatment with 1 mg/kg, but not 0.1 mg/kg, significantly increased striatal neuronal counts compared to untreated animals (c). Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$; n.s.=no statistical significance.

3.5. References

Akao, Y., Maruyama, W., Shimizu, S., Yi, H., Nakagawa, Y., Shamoto-Nagai, M., Youdim, M.B.H., Tsujimoto, Y., Naoi, M., 2002. Mitochondrial permeability transition mediates apoptosis induced by N-methyl(R)salsolinol, an endogenous neurotoxin, and is inhibited by Bcl-2 and rasagiline, N-propargyl-1(R)-aminoindan. J Neurochem. 82**,** 913-23.

Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J., Martin, J.B., 1986. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 321**,** 168-71.

Beal, M.F., Brouillet, E., Jenkins, B., Henshaw, R., Rosen, B., Hyman, B.T., 1993. Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. J Neurochem. 61**,** 1147-50.

Eliash, S., Speiser, Z., Cohen, S., 2001. Rasagiline and its (S) enantiomer increase survival and prevent stroke in salt-loaded stroke-prone spontaneously hypertensive rats. Journal of neural transmission (Vienna, Austria : 1996). 108**,** 909-23.

Eliash, S., Dror, V., Cohen, S., Rehavi, M., 2009. Neuroprotection by rasagiline in thiamine deficient rats. Brain Res. 1256**,** 138-48.

Fernandes, H.B., Baimbridge, K.G., Church, J., Hayden, M.R., Raymond, L.A., 2007. Mitochondrial sensitivity and altered calcium handling underlie enhanced NMDA-induced apoptosis in YAC128 model of Huntington's disease. J Neurosci. 27**,** 13614-23.

Finberg, J.P., Tenne, M., Youdim, M.B., 1981. Tyramine antagonistic properties of AGN 1135, an irreversible inhibitor of monoamine oxidase type B. British Journal of Pharmacology. 73**,** 65-74.

Finberg, J.P., Takeshima, T., Johnston, J.M., Commissiong, J.W., 1998. Increased survival of dopaminergic neurons by rasagiline, a monoamine oxidase B inhibitor. Neuroreport. 9**,** 703-7.

Graham, R.K., Deng, Y., Slow, E.J., Haigh, B., Bissada, N., Lu, G., Pearson, J., Shehadeh, J., Bertram, L., Murphy, Z., Warby, S.C., Doty, C.N., Roy, S., Wellington, C.L., Leavitt, B.R., Raymond, L.A., Nicholson, D.W., Hayden, M.R., 2006. Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell. 125**,** 1179-91.

Group, P.S., 2002. A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. Arch Neurol. 59**,** 1937-43.

Group, P.S., 2005. A randomized placebo-controlled trial of rasagiline in levodopa-treated patients with Parkinson disease and motor fluctuations: the PRESTO study. Arch Neurol. 62**,** 241-8.

Group, T.H.s.D.C.R., 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**,** 971-83.

Henshaw, R., Jenkins, B.G., Schulz, J.B., Ferrante, R.J., Kowall, N.W., Rosen, B.R., Beal, M.F., 1994. Malonate produces striatal lesions by indirect NMDA receptor activation. Brain Res. 647**,** 161-6.

Hoyt, K.R., Gallagher, A.J., Hastings, T.G., Reynolds, I.J., 1997. Characterization of hydrogen peroxide toxicity in cultured rat forebrain neurons. Neurochem Res. 22**,** 333-40.

Kremer, B., Weber, B., Hayden, M.R., 1992. New insights into the clinical features, pathogenesis and molecular genetics of Huntington disease. Brain Pathol. 2**,** 321-35.

Kupsch, A., Sautter, J., Götz, M.E., Breithaupt, W., Schwarz, J., Youdim, M.B., Riederer, P., Gerlach, M., Oertel, W.H., 2001. Monoamine oxidase-inhibition and MPTP-induced neurotoxicity in the non-human primate: comparison of rasagiline (TVP 1012) with selegiline. Journal of neural transmission (Vienna, Austria : 1996). 108**,** 985-1009.

Mandel, S., Weinreb, O., Amit, T., Youdim, M.B.H., 2005. Mechanism of neuroprotective action of the anti-Parkinson drug rasagiline and its derivatives. Brain Res Brain Res Rev. 48**,** 379-87.

Maruyama, W., Yamamoto, T., Kitani, K., Carrillo, M.C., Youdim, M., Naoi, M., 2000. Mechanism underlying anti-apoptotic activity of a (-)deprenyl-related propargylamine, rasagiline. Mech Ageing Dev. 116**,** 181-91.

Maruyama, W., Youdim, M.B., Naoi, M., 2001. Antiapoptotic properties of rasagiline, N-propargylamine-1(R)-aminoindan, and its optical (S)-isomer, TV1022. Annals of the New York Academy of Sciences. 939**,** 320-9.

Metzler, M., Gan, L., Wong, T.P., Liu, L., Helm, J., Liu, L., Georgiou, J., Wang, Y., Bissada, N., Cheng, K., Roder, J.C., Wang, Y.T., Hayden, M.R., 2007. NMDA receptor function and NMDA receptor-dependent phosphorylation of huntingtin is altered by the endocytic protein HIP1. J Neurosci. 27**,** 2298-308.

Mytilineou, C., Radcliffe, P., Leonardi, E.K., Werner, P., Olanow, C.W., 1997. L-deprenyl protects mesencephalic dopamine neurons from glutamate receptor-mediated toxicity in vitro. J Neurochem. 68**,** 33-9.

Panov, A.V., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., Burke, J.R., Strittmatter, W.J., Greenamyre, J.T., 2002. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat. Neurosci. 5**,** 731-6.

Patel, S.V., Tariot, P.N., Asnis, J., 1996. L-Deprenyl augmentation of fluoxetine in a patient with Huntington's disease. Annals of clinical psychiatry : official journal of the American Academy of Clinical Psychiatrists. 8**,** 23-6.

Pouladi, M., Bezprozvanny, I., Raymond, L.A., Hayden, M., 2006. Molecular Pathogenesis of Huntington's Disease: The Role of Excitotoxicity. Genetic Instabilities and Neurological Diseases.

R. Hayden, M., 1981. Huntington's Chorea.

Rascol, O., Brooks, D.J., Melamed, E., Oertel, W., Poewe, W., Stocchi, F., Tolosa, E., group, L.s., 2005. Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. Lancet. 365**,** 947-54.

Roze, E., Saudou, F., Caboche, J., 2008. Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. Curr Opin Neurol. 21**,** 497-503.

Schulz, J.B., Matthews, R.T., Klockgether, T., Dichgans, J., Beal, M.F., 1997. The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative diseases. Mol Cell Biochem. 174**,** 193-7.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12**,** 1555-67.

Speiser, Z., Katzir, O., Rehavi, M., Zabarski, T., Cohen, S., 1998. Sparing by rasagiline (TVP-1012) of cholinergic functions and behavior in the postnatal anoxia rat. Pharmacol Biochem Behav. 60**,** 387-93.

Speiser, Z., Mayk, A., Eliash, S., Cohen, S., 1999. Studies with rasagiline, a MAO-B inhibitor, in experimental focal ischemia in the rat. Journal of neural transmission (Vienna, Austria : 1996). 106**,** 593-606.

Speiser, Z., Mayk, A., Litinetsky, L., Fine, T., Nyska, A., Blaugrund, E., Cohen, S., 2007. Rasagiline is neuroprotective in an experimental model of brain ischemia in the rat. Journal of neural transmission (Vienna, Austria : 1996). 114**,** 595-605.

Stefanova, N., Poewe, W., Wenning, G.K., 2008. Rasagiline is neuroprotective in a transgenic model of multiple system atrophy. Experimental Neurology. 210**,** 421-7.

Tang, T.-S., Slow, E., Lupu, V., Stavrovskaya, I.G., Sugimori, M., Llinás, R., Kristal, B.S., Hayden, M.R., Bezprozvanny, I., 2005. Disturbed Ca2+ signaling and apoptosis of medium spiny neurons in Huntington's disease. Proc Natl Acad Sci USA. 102**,** 2602-7.

Tang, T.-S., Chen, X., Liu, J., Bezprozvanny, I., 2007. Dopaminergic signaling and striatal neurodegeneration in Huntington's disease. J Neurosci. 27**,** 7899-910.

Van Raamsdonk, J.M., Pearson, J., Rogers, D.A., Lu, G., Barakauskas, V.E., Barr, A.M., Honer, W.G., Hayden, M.R., Leavitt, B.R., 2005a. Ethyl-EPA treatment improves motor

dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Experimental Neurology. 196**,** 266-72.

Van Raamsdonk, J.M., Pearson, J., Slow, E.J., Hossain, S.M., Leavitt, B.R., Hayden, M.R., 2005b. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci. 25**,** 4169-80.

Whittemore, E.R., Loo, D.T., Watt, J.A., Cotman, C.W., 1995. A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. Neuroscience. 67**,** 921-32.

Youdim, M.B.H., Bar Am, O., Yogev-Falach, M., Weinreb, O., Maruyama, W., Naoi, M., Amit, T., 2005. Rasagiline: neurodegeneration, neuroprotection, and mitochondrial permeability transition. J. Neurosci. Res. 79**,** 172-9.

Zeron, M.M., Fernandes, H.B., Krebs, C., Shehadeh, J., Wellington, C.L., Leavitt, B.R., Baimbridge, K.G., Hayden, M.R., Raymond, L.A., 2004. Potentiation of NMDA receptor-mediated excitotoxicity linked with intrinsic apoptotic pathway in YAC transgenic mouse model of Huntington's disease. Mol Cell Neurosci. 25**,** 469-79.

4. Treatment with the NMDA receptor antagonist memantine in combination with the propargylamine rasagiline improves motor function and rescues striatal deficits in the YAC128 mouse model of Huntington disease[3]

4.1. Introduction

Huntington disease (HD) is a hereditary, age-dependent neurodegenerative disease caused by a CAG trinucleotide expansion within the *IT15* gene, which encodes huntingtin (htt). Patients carrying the *HD* mutation develop a range of neurological symptoms that include cognitive deficits, affective disturbances, and difficulties in movement and motor coordination. Neuropathologically, degeneration of the basal ganglia with selective loss of medium spiny neurons of the striatum constitutes the principal feature of the disease (Hayden, 1981). Despite significant advances in understanding the underlying pathogenic processes, the disease remains without a cure with death typically occurring 10-15 years following onset.

The aim of this study was to assess the potential of memantine given in combination with rasagiline as a therapy in HD. Memantine is a clinically well-tolerated NMDA receptor antagonist that has been approved for the treatment of patients with moderate to severe Alzheimer's disease (Reisberg et al., 2003; Tariot et al., 2004), and is currently being evaluated as a possible treatment for a number of conditions including depression, anxiety, chronic pain, HIV-associated dementia, and ALS (Lipton, 2006). Rasagiline is a selective inhibitor of monoamine oxidase type B (MAO-B) (Finberg et al., 1981) clinically approved for the treatment

[3] A version of this chapter is in preparation for submission. Pouladi MA, Dar Santos R, Zapala M, Xie Y, Hayden MR. Treatment with the NMDA receptor antagonist memantine in combination with the propargylamine rasagiline improves motor function and rescues striatal deficits in the YAC128 mouse model of Huntington disease.

of Parkinson's disease (PD) both as a monotherapy (Group, 2002) and as adjunct therapy to levodopa (Group, 2005; Rascol et al., 2005), and which has been shown to protect against a number of neurotoxic stimuli (Mandel et al., 2005; Youdim et al., 2005).

We have previously examined the therapeutic potential of memantine and rasagiline, each given alone, in the transgenic YAC128 HD animals (Chapters 4 and 5, respectively). The YAC128 animals express human mutant htt with a 128 CAG trinucleotide repeats and develop progressive motor dysfunction and selective striatal deficits, recapitulating key features of the human condition (Slow et al., 2003). We demonstrated that treatment with 1 mg/kg of memantine results in improvements in motor function late, but not early, in the disease phenotype and rescues the striatal deficits in the YAC128 HD mice (Chapter 4). We further demonstrated that treatment with 0.1 mg/kg of rasagiline results in improvements in motor function both early and late in the disease phenotype, but does not rescue the striatal deficits in the YAC128 HD animals (Chapter 5).

The concurrent use of multiple therapeutic agents to attain greater benefit holds great promise and has indeed been employed successfully in combating numerous disease conditions including cancer, HIV, and rheumatoid arthritis (Dimopoulos et al., 2007; Van Cutsem et al., 2009; Montaner et al., 1998; Tugwell et al., 1995). By targeting different pathogenic pathways simultaneously, greater benefit may be achieved than that provided by monotherapy.

In this study, we investigate whether treatment with memantine and rasagiline in combination could provide early and sustained improvement in motor function and afford neuroprotection in the transgenic YAC128 mouse model of HD.

4.2. Materials and methods

Animals

Male and female YAC128 mice expressing expanded human huntingtin with 128 CAG repeats and WT littermates maintained on the FVB/N strain (Charles River, Wilmington, MA) were used for these experiments (Slow et al., 2003). Mice were housed singly or in pairs in duplex cages with littermates of mixed genotype and maintained under a 12 L:12 D light cycle (lights on at 2300) in a clean facility and given free access to food and water. Experimenters were blind to the genotype of the mice. All experiments were performed with the approval of the animal care committee at the University of British Columbia.

Drug treatment

A dose of 1 mg/kg of memantine (Figure 4.1a) and 0.1 mg/kg of rasagiline (Figure 4.1b), administered in the drinking water, was chosen for the trial based our findings in the single-drug trials of memantine (Chapter 2) and rasagiline (Chapter 3). For administration in the drinking water, water consumption of individual cages was monitored on a biweekly basis along with animal body weights. The concentrations of drug solutions for each cage were then adjusted accordingly. The drug solutions were replaced twice/week and were provided ad libitum.

Motor function assessment

Training and baseline testing for motor function tasks was carried out at 2 months of age. Testing took place during the dark cycle and was curried out every two months between 2 and 12 months of age. Motor co-ordination and balance was assessed using accelerating rotarod task (UGO Basile, Comerio, Italy). In this task, the rotarod accelerated from 5 revolutions per minute (RPM) to 40 RPM over 5 minutes. Performance was assessed by the amount of time that a mouse could remain running on the rotarod; the maximum scores is 300 seconds. During training, mice were

given three trials per day for three consecutive days. Rotarod scores are the average of three trials spaced 2 h apart.

Brain sample preparation

Mice were injected with heparin followed by terminal anesthesia with intraperitoneally-injected 2.5% avertin. The animals were perfused with 3% paraformaldehyde with 0.6% glutaraldehyde in phosphate-buffered saline (PBS). Brains were left in the skulls for 24 hrs in 3% paraformaldehyde, then removed and stored in PBS. After weighing, the brains were transferred to a 30% sucrose solution containing 0.08% sodium azide in PBS. They were then frozen on dry ice, mounted with Tissue-TEK O.C.T. compound (Sakura, Torrance, CA), and sliced coronally into 25 μm sections on a cryostat (Microm HM 500M, Richard-Allan Scientific, Calamazoo, MI). The sections are collected and stored in PBS with 0.08% sodium azide at 4 degrees.

Neuropathological analysis

A series of 25 μm-thick coronal sections spaced 200 μm apart spanning the striatum were stained with NeuN antibody (1:100; Chemicon, Temecula, CA) overnight at room temperature, followed by incubation with biotinylated anti-mouse antibody (1:200; Vector Laboratories, Burlingame, CA). The signal was amplified with an ABC Elite kit (Vector) and detected with diaminobenzidine (DAB; Pierce, Rockford, IL). Striatal volume was determined from a series of mounted sections using StereoInvestigator software (Microbrightfield, Williston, VT). Briefly, striatal volumes were determined by tracing the perimeter of the striatum in serial sections spanning the striatum.

Statistical analysis

Data are expressed as means±SEM. Whenever suitable, results were interpreted using one-way ANOVA with a Student-Newman-Keuls (SNK) post-hoc test. Pairwise comparisons between

genotypes/treatments at individual time points were assessed with a Student's t-test. Differences were considered statistically significant when $P < 0.05$.

4.3. Results

Treatment with a combination of memantine and rasagiline improves motor function in the YAC128 HD animals

To examined the effect of treatment with 1 mg/kg of memantine and 0.1 mg/kg of rasagiline in combination (combo) on motor function, YAC128 and WT animals were treated starting at 2 months of age and their performance along with that of untreated animals on the accelerating rotarod test was assessed at 2 (baseline), 4, 6, 8, and 12 months of age. Untreated YAC128 animals had a significantly lower performance, as signified by decreased latency to fall, at 4, 6, 8, and 12 months of age compared to WT (Figure 4.2a). Combo-treated YAC128 animals had a significantly lower performance at 8 and 12 months compared to WT, but were not statistically different than WT at 4 and 6 months of age. Furthermore, combo-treated YAC128 animals had a significantly improved performance compared to untreated YAC128 animals at 4, 6, and 12 months of age (Figure 4.2a,b). The performance of combo-treated WT animals was significantly improved at 4, 6, 8, and 12 months of age compared to untreated WT (Figure 4.2c,d).

Treatment with a combination of memantine and rasagiline rescues striatal neuropathology in the YAC128 HD animals

To investigate the effect of combo-treatment on striatal pathology in the YAC128 HD animals, striatal volume and neuronal counts were assessed in treated animals at 12 months of age. Untreated YAC128 animals had a significantly lower striatal volume compared to untreated WT (Figure 4.3a). Combo-treated YAC128 HD animals had a significantly larger striatal volume compared to untreated YAC128 HD animals. Furthermore, there was no significant difference in striatal volume between combo-treated YAC128 HD animals and WT (Figure 4.3a).

Neuronal (NeuN-positive) counts were also assessed. Untreated YAC128 animals had significantly lower neuronal counts compared to WT (Figure 4.3b). Combo-treated YAC128 HD

animals had higher neuronal counts compared to untreated YAC128 HD animals, although the difference did not reach statistical significance (P=0.089) (Figure 4.3b). Furthermore, there was no statistical difference in neuronal counts between combo-treated YAC128 HD animals and WT (Figure 4.3b).

There were no differences in striatal volume or neuronal counts between untreated and combo-treated WT animals (Figure 4.4a,b).

4.4. Discussion

The aim of this study was to evaluate the effect of treatment with memantine in combination with rasagiline on the motor and neuropathological deficits in YAC128 HD mice. Our findings demonstrate that treatment with a combination of memantine and rasagiline improves motor function both early and late in the disease phenotype and rescues striatal deficits in the YAC128 HD animals.

Excessive glutamate receptor signaling, particularly via the NMDA-type glutamate receptors, in conjunction with aberrant mitochondrial calcium handling are thought to lead to disrupted intracellular calcium homeostasis and ultimately cell death in a process termed excitotoxicity. Excitotoxicity is thought to play a key role in the pathogenesis of HD and strategies aimed at attenuating this enhanced excitotoxic cell death hold therapeutic potential.

We have previously shown that memantine, an uncompetitive NMDA receptor antagonist, can attenuate excitotoxic cell death in striatal medium spiny neurons and that long-term treatment with a low-dose of memantine (1 mg/kg) leads to improvements in motor function that are seen late in the disease phenotype (12 months of age) (Chapter 2) and rescues the striatal pathology in YAC128 HD animals. We have further shown that rasagiline, an MAO-B inhibitor with neuroprotective properties, can attenuate excitotoxic striatal lesions and that long-term treatment with a low-dose of rasagiline (0.1 mg/kg) leads to early and sustained improvements in motor function (4, 6, 8, and 12 months of age) but no rescue of striatal pathology in the YAC128 HD animals (Chapter 3). Our findings in the present study indicate that treatment with a combination of memantine and rasagiline leads to improvements in motor function both early (6 months) and late (12 months) in the disease phenotype, and rescue of the striatal pathology in the YAC128 HD animals (Figure 4.5).

That early and sustained benefit is observed with the combination treatment may reflect the simultaneous targeting of the excitotoxic process at multiple points. While memantine is known to attenuate excitotoxicity by moderating calcium influx via NMDA receptors (Lipton, 2006), rasagiline is thought to act by modulating the mitochondrial membrane potential and preventing the induction of the mitochondrial permeability pore (mPT) (Youdim et al., 2005). Their combined action likely leads to a further decrease in excitotoxicity than with either compound alone. Indeed, given the multiplicity of pathogenic disturbances identified in HD, use of combinations of therapeutic agents with complementary cellular targets may be necessary to achieve maximal improvement and benefit in HD particularly, and in other neurodegenerative diseases in general (Youdim and Buccafusco, 2005).

As the trials of memantine, rasagiline, and memantine in combination with rasagiline were carried it sequentially and not in parallel, our ability to draw conclusions on whether the effects of the combination treatment are additive or synergistic is limited. Indeed, a four-arm trial that compares memantine-alone, rasagiline-alone, and a memantine-rasagiline combination to untreated animals would be required to address this question. Furthermore, there is a possibility of drug interaction between memantine and rasagiline that may impact on their respective pharmacodynamic and/or pharmacokinetic properties, and their efficacy as a consequence. Therefore, trial of combinations of different doses of the two compounds may be necessary to arrive at the optimal combination of doses that provide maximal benefit for these drugs.

In summary, we demonstrate that treatment with a combination of memantine and rasagiline yields early and sustained improvements in motor function and rescuing striatal deficits in the YAC128 HD mice.

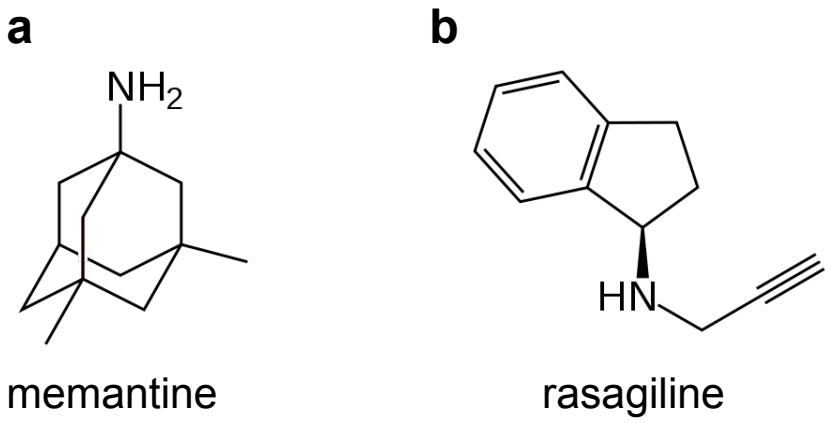


Figure 4.1 Chemical structures of memantine and rasagiline

Chemical structure of (**a**) memantine, 3,5-Dimethyl-tricyclo[3.3.1.13,7]decan-1-amine (CAS 19982-08-2), and (**b**) rasagiline, (1R)-N-prop-2-ynyl-2,3-dihydro-1H-inden-1-amine (CAS 161735-79-1).

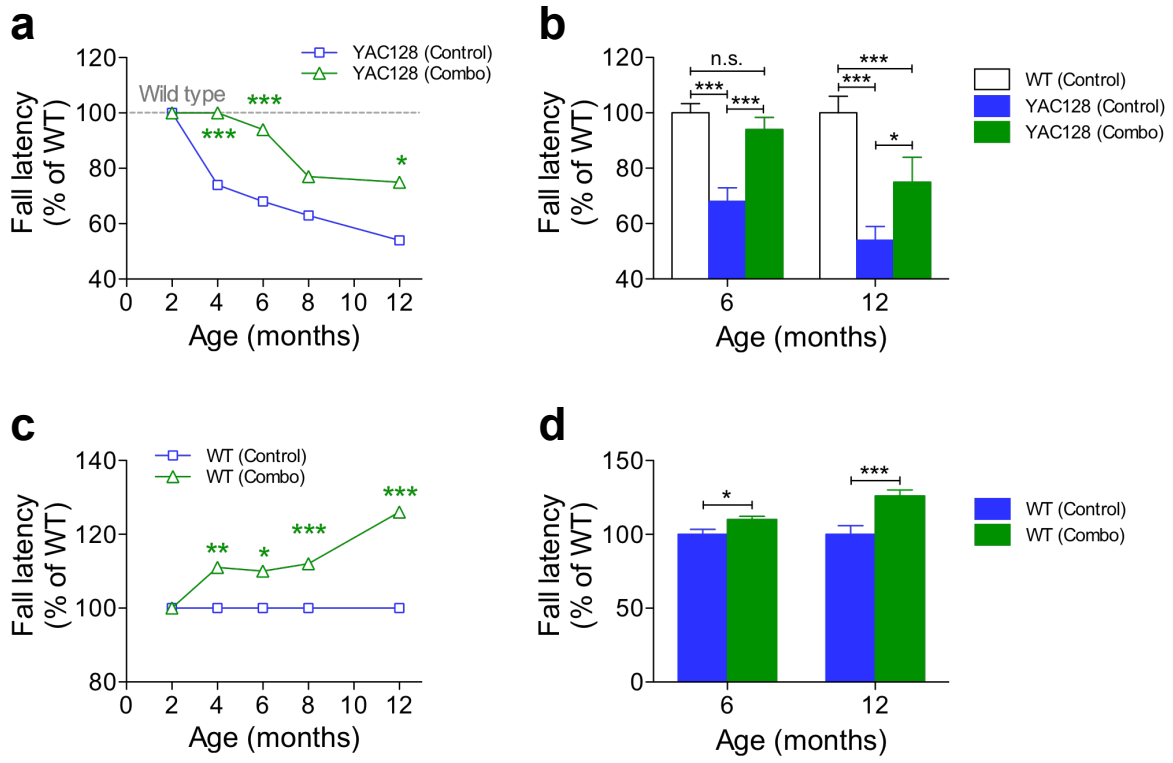


Figure 4.2 Treatment with a combination of memantine and rasagiline improves motor function in the transgenic YAC128 HD animals

YAC128 and WT animals were treated with a combination of 1 mg/kg memantine and 0.1 mg/kg rasagiline (combo), and their motor function along with that of untreated YAC128 and WT animals was assessed at 2 (baseline), 4, 6, 8, and 12 months of age. (**a, b**) Untreated YAC128 animals had a significantly lower performance at 4, 6, 8, and 12 months of age compared to WT. Combo-treated YAC128 animals had a significantly lower performance at 8 and 12 months compared to WT, but were not statistically different than WT at 4 and 6 months of age. Furthermore, combo-treated YAC128 animals had a significantly improved performance compared to untreated YAC128 animals at 4, 6, and 12 months of age. (**c, d**) The performance of combo-treated WT animals was significantly improved at 4, 6, 8, and 12 months of age compared to untreated WT. Data represent mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared to untreated animals of the same genotype, unless otherwise indicated.

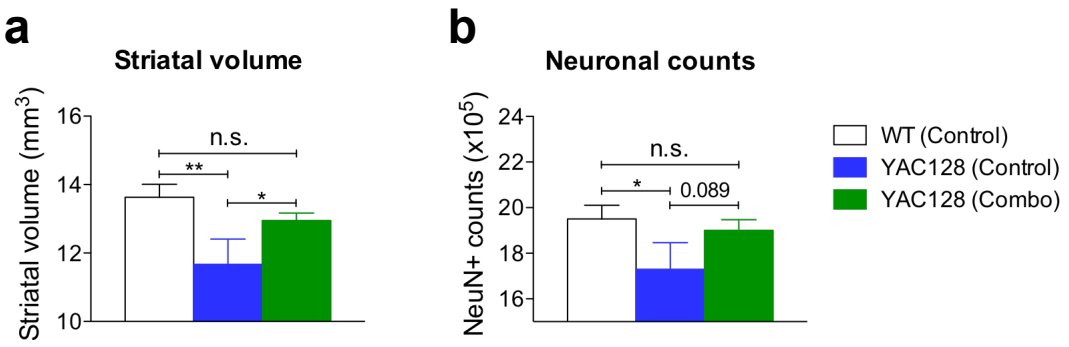


Figure 4.3 Treatment of YAC128 HD animals with a combination of memantine and rasagiline rescues striatal pathology

To determine the effect of treatment with a combination of 1 mg/kg memantine and 0.1 mg/kg rasagiline (combo) on neuropathology in YAC128 HD animals, 2 months old YAC128 animals were treated for 10 months and striatal volume and neuronal counts were assessed at 12 months of age. (**a**) Untreated YAC128 animals had a significantly lower striatal volume compared to WT. Combo-treated YAC128 HD animals had a significantly larger striatal volume compared to untreated YAC128 HD animals. Furthermore, there was no statistical difference in striatal volume between combo-treated YAC128 HD animals and WT. (**b**) Untreated YAC128 animals had a significantly lower neuronal count compared to WT. Combo-treated YAC128 HD animals had a higher neuronal count compared to untreated YAC128 HD animals, although the difference did not reach statistical significance (P=0.089). Furthermore, there was no statistical difference in neuronal counts between combo-treated YAC128 HD animals and WT. Data represent mean ± s.e.m. *, $P < 0.05$; ** $P < 0.01$; n.s.=no statistical significance.

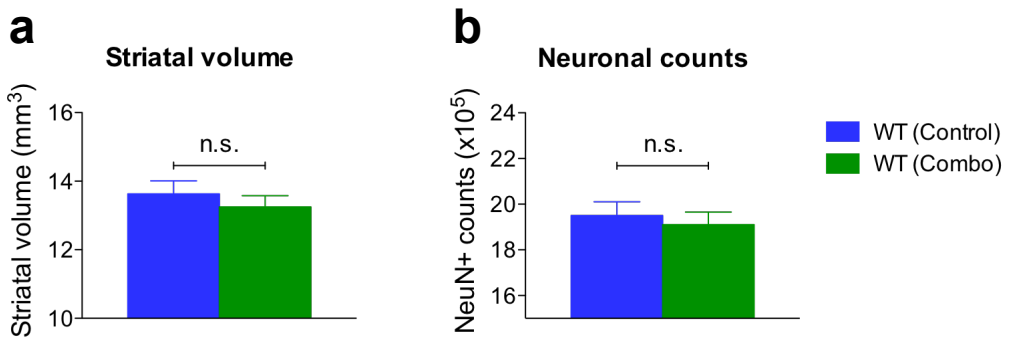


Figure 4.4 Treatment of WT animals with a combination of memantine and rasagiline has no effect on striatal volume and neuronal counts

Effects of treatment with a combination of 1 mg/kg memantine and 0.1 mg/kg rasagiline (combo) for 10-months starting at 2 months of age on striatal volume and striatal neuronal counts were examined in WT animals. There were no statistically significant differences in striatal volume (**a**) or neuronal counts (**b**) between combo-treated and untreated WT animals. Data represent mean ± s.e.m; n.s.=no statistical significance.

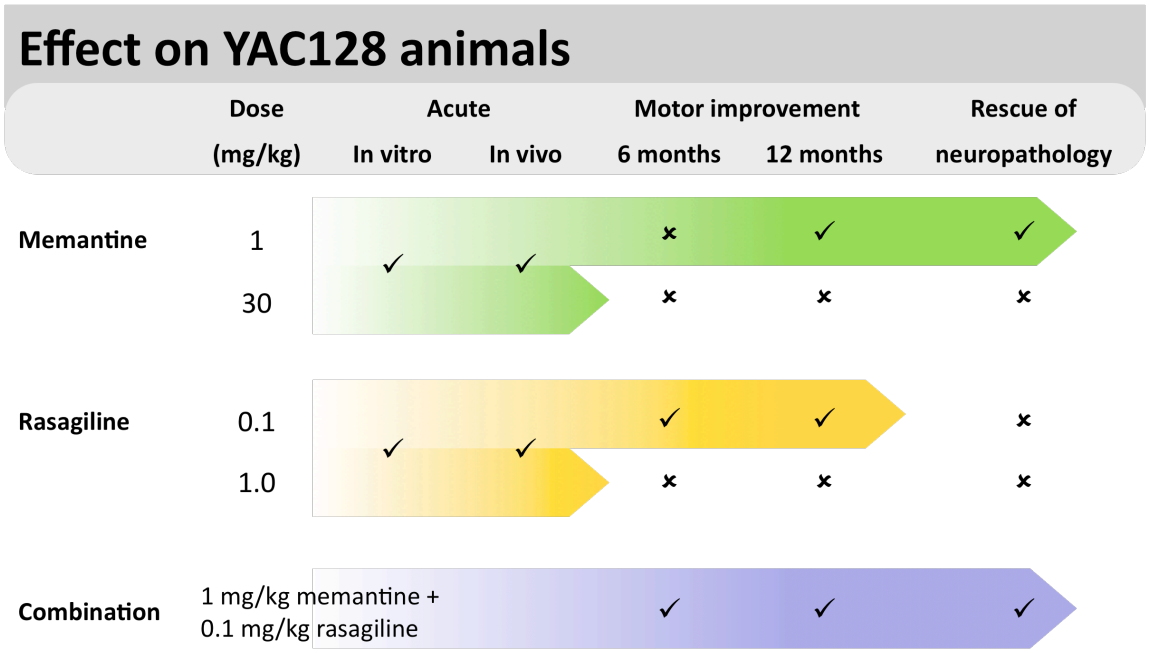


Figure 4.5 Treatment with a combination of memantine and rasagiline leads to early and sustained improvements in motor function and rescue of striatal pathology in YAC128 HD mice

Treatment of YAC128 animals with 1 mg/kg of memantine improves motor function late, but not early, in the disease phenotype and rescues striatal deficits. Treatment of YAC128 animals with 0.1 mg/kg rasagiline improves motor function both early and late in the disease phenotype, but does not rescue striatal deficits. Treatment with a combination of rasagiline and memantine results in early and late improvements in motor function and rescue of striatal deficits.

4.5. References

Dimopoulos, M., Spencer, A., Attal, M., Prince, H.M., Harousseau, J.L., Dmoszynska, A., San Miguel, J., Hellmann, A., Facon, T., Foa, R., Corso, A., Masliak, Z., Olesnyckyj, M., Yu, Z., Patin, J., Zeldis, J.B., Knight, R.D., 2007. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 357, 2123-32.

Finberg, J.P., Tenne, M., Youdim, M.B., 1981. Tyramine antagonistic properties of AGN 1135, an irreversible inhibitor of monoamine oxidase type B. British Journal of Pharmacology. 73**,** 65-74.

Group, P.S., 2002. A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. Arch Neurol. 59**,** 1937-43.

Group, P.S., 2005. A randomized placebo-controlled trial of rasagiline in levodopa-treated patients with Parkinson disease and motor fluctuations: the PRESTO study. Arch Neurol. 62**,** 241-8.

Lipton, S.A., 2006. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov. 5**,** 160-70.

Mandel, S., Weinreb, O., Amit, T., Youdim, M.B.H., 2005. Mechanism of neuroprotective action of the anti-Parkinson drug rasagiline and its derivatives. Brain Res Brain Res Rev. 48**,** 379-87.

Montaner, J.S., Reiss, P., Cooper, D., Vella, S., Harris, M., Conway, B., Wainberg, M.A., Smith, D., Robinson, P., Hall, D., Myers, M., Lange, J.M., 1998. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA. 279, 930-7.

R. Hayden, M., 1981. Huntington's Chorea.

Rascol, O., Brooks, D.J., Melamed, E., Oertel, W., Poewe, W., Stocchi, F., Tolosa, E., group, L.s., 2005. Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. Lancet. 365**,** 947-54.

Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., Möbius, H.J., Group, M.S., 2003. Memantine in moderate-to-severe Alzheimer's disease. N Engl J Med. 348**,** 1333-41.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12**,** 1555-67.

Tariot, P.N., Farlow, M.R., Grossberg, G.T., Graham, S.M., McDonald, S., Gergel, I., Group, M.S., 2004. Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial. JAMA. 291**,** 317-24.

Tugwell, P., Pincus, T., Yocum, D., Stein, M., Gluck, O., Kraag, G., McKendry, R., Tesser, J., Baker, P., Wells, G., 1995. Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. The Methotrexate-Cyclosporine Combination Study Group. N Engl J Med. 333, 137-41.

Van Cutsem, E., Kohne, C.H., Hitre, E., Zaluski, J., Chang Chien, C.R., Makhson, A., D'Haens, G., Pinter, T., Lim, R., Bodoky, G., Roh, J.K., Folprecht, G., Ruff, P., Stroh, C., Tejpar, S., Schlichting, M., Nippgen, J., Rougier, P., 2009. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 360, 1408-17.

Youdim, M.B.H., Bar Am, O., Yogev-Falach, M., Weinreb, O., Maruyama, W., Naoi, M., Amit, T., 2005. Rasagiline: neurodegeneration, neuroprotection, and mitochondrial permeability transition. J. Neurosci. Res. 79**,** 172-9.

Youdim, M.B.H., Buccafusco, J.J., 2005. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. Trends Pharmacol Sci. 26**,** 27-35.

5. Treatment with arimoclomol does not lead to up-regulation of heat shock proteins or rescue of the behavioural and striatal deficits in the YAC128 mouse model of Huntington disease[4]

5.1. Introduction

Huntington Disease (HD) is an inherited neurological disorder characterized by loss of motor coordination, cognitive dysfunction, and psychiatric disturbances (Hayden, 1981). The cardinal neuropathological feature of HD is loss of medium spiny neurons within the striatum and decreased striatal volume. The disease is progressive in nature resulting in death 15-20 years after onset, and there is currently no cure or treatment to interfere in the course of the illness.

Considerable evidence accumulated over the past 20 years points to excitotoxicity as a likely pathogenic mechanism contributing to HD (Fan and Raymond, 2007; Pouladi et al., 2006). Indeed, intrastriatal injections of the excitatory NMDA receptor agonist quinolinic acid (QA) produce lesions that mimic many of the neurochemical and histopathological features of HD (Beal et al., 1986; Beal et al., 1988; Beal et al., 1991; Ferrante et al., 1985) and are associated with HD-like behavioural deficits (Furtado and Mazurek, 1996; Popoli et al., 1994; Shear et al., 1998). Moreover, human studies demonstrate enhanced expression of NMDA glutamate receptors in striatal medium-sized spiny neurons selectively lost in HD patients compared to the spared interneurons (Landwehrmeyer et al., 1995; Young et al., 1988). Studies in vitro and in transgenic rodent models of HD revealed enhanced susceptibility to excitotoxic cell death in the presence of mutant huntingtin, further implicating excitotoxicity in the pathogenesis of HD (Fan

[4] A version of this chapter is in preparation for submission. Pouladi MA, Carroll JB, Dar Santos R, Bertram LN, and Hayden MR. Treatment with arimoclomol does not lead to up-regulation of heat shock proteins or rescue of the behavioural and striatal deficits in the YAC128 mouse model of Huntington disease.

and Raymond, 2007). As such, strategies aimed at modulating excitotoxicity have been proposed as being potentially therapeutic in HD.

Heat shock proteins (HSPs) are a family of molecular chaperones that form part of the cellular stress response. HSPs have been shown to be neuroprotective in acute excitotoxicity models (Dedeoglu et al., 2002) and in models of polyglutamine-induced neurodegenerative disease (Fujikake et al., 2008; Fujimoto et al., 2005; Katsuno et al., 2005; Perrin et al., 2007; Vacher et al., 2005; Zhang and Sarge, 2007). Hence, induction of HSPs may reduce the susceptibility to excitotoxic cell death thought to contribute to HD and delay the onset or progression of HD.

The expression of a number of heat shock proteins has been shown to be regulated by the transcription factor Hsf-1 (Ohtsuka and Suzuki, 2000). Increased transgenic expression or pharmacological induction of Hsf-1 results in increased expression of HSPs such as HSP70 and HSP90 and is associated with improvements in the phenotype of several animal models of neurodegenerative disease (Fujimoto et al., 2005; Hay et al., 2004; Katsuno et al., 2005; Ohtsuka and Suzuki, 2000; Zhang and Sarge, 2007).

Arimoclomol is member of a family of hydroxylamine derivatives that have been shown to bind to and prolong the activation of Hsf-1. This enhanced activation of Hsf-1 results in prolonged binding of Hsf-1 to a region in the promoter of heat shock genes known as the heat shock response element, allowing increased expression of heat shock protein transcripts (Hargitai et al., 2003; Kieran et al., 2004). Treatment with arimoclomol has been shown to be neuroprotective in a mouse model of ALS (Kalmar et al., 2008; Kieran et al., 2004) and is well tolerated and safe when administered to ALS patients (Cudkowicz et al., 2008).

The aim of this study was to assess whether treatment with arimoclomol could provide neuroprotection in the acute lesional quinolinic acid (QA) model of HD and the transgenic YAC128 mouse model of HD.

5.2. Materials and methods

Animals

Male and female YAC128 mice expressing expanded human huntingtin with 128 CAG repeats and WT littermates maintained on the FVB/N strain (Charles River, Wilmington, MA) were used for these experiments (Slow et al., 2003). Mice were housed singly or in pairs in duplex cages with littermates of mixed genotype and maintained under a 12 L:12 D light cycle (lights on at 2300) in a clean facility and given free access to food and water. Experimenters were blind to the genotype of the mice. All experiments were performed with the approval of the animal care committee at the University of British Columbia.

Quinolinic Acid Lesion

To induce NMDAR-mediated striatal lesions, 3 months old mice were anesthetized with avertin (2.5%) and placed in a stereotaxic instrument. Coordinates for the unilateral striatal injections were as follows: 0.8 mm posterior to Bregma, 1.8 mm lateral from the midline, and 3.5 mm below the dorsal surface of the neocortex. Quinolinic acid (QA) was injected (1 μL) over a 2 min period. For the acute treatment study, FVB/N mice received an intraperitoneal injection of either vehicle (PBS) or arimoclomol (40 mg/kg) and were injected intrastriatally with 25 nmol of QA 30 min later. For the short-term treatment study, 3-months old YAC128 animals were treated with 200 mg/kg arimoclomol for 14 days with an intrastriatal injection of 25 nmol QA on day 7.

Seven days after the intrastriatal QA injection, animals were injected with heparin and terminally anesthetized with intraperitoneally-injected 2.5% avertin. The brains were extracted and treated as described in the brain sample preparation section. For each mouse, coronal 25 μm sections spaced 200 μm apart throughout the striatum were stained with FluoroJade B (Histo-Chem, Jefferson, Arkansas), a fluorescent stain that labels degenerating neurons in fixed brain sections,

and the total striatal lesion volume was determined using unbiased techniques and StereoInvestigator software (Microbrightfield, Williston, VT).

Drug treatment

A dose of 80 mg/kg, administered in the drinking water, was chosen for the trial based on the recommendation of CytRx Inc, the developer of arimoclomol (Dr. Jack Barber, CytRx Inc, personal communication). For administration in the drinking water, water consumption of individual cages was monitored on a biweekly basis along with animal body weights. The concentrations of drug solutions for each cage were then adjusted accordingly. The drug solutions were replaced twice/week and were provided ad libitum.

Immunoblot analysis

Immunoblots were performed on whole brain tissue samples frozen immediately following euthanasia. One total brain hemisphere was homogenized in 1 ml/100 mg tissue lysis buffer (20 mM Tris/HCl pH 7.2, 10 mM MgCl2, 0.5 mM EDTA, 322 mM sucrose and 1x complete protease inhibitor cocktail (Roche)) with an automated tissue homogenizer for 30 sec. Protein lysates were incubated on ice for 1h, sonicated for 30 sec at 30% output, passaged through a 25G needle and separated on an SDS-PAGE gels. Following transfer to a nitrocellulose membrane, blots were immunostained overnight at 4°C with primary antibodies. Monoclonocal mouse anti-HSP70 antibody (SPA-810), and polyclonal rabbit anti-HSP90 antibody (SPA-846) were from Stressgen (Victoria, BC). Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was from Chemicon. Horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham Biosciences, Monmouth Junction, NJ, USA) were used to visualize the blots.

Motor function assessment

Training and baseline testing for motor function tasks was carried out at 2 months of age. Testing took place during the dark cycle and was curried out every two months between 2 and 12 months of age. Motor co-ordination and balance was assessed using accelerating and fixed rotarod tasks (UGO Basile, Comerio, Italy). In the accelerating task, the rotarod accelerated from 5 revolutions per minute (RPM) to 40 RPM over 5 minutes. In the fixed task, the rotarod revolved at 24 RPM. Performance in the rotarod tasks was assessed by the amount of time that a mouse could remain running on the rotarod; the maximum scores in the accelerating and fixed tasks are 300 seconds and 60 seconds, respectively. During training, mice were given three trials per day for three consecutive days. Rotarod scores are the average of three trials spaced 2 h apart.

Brain sample preparation

Mice were injected with heparin followed by terminal anesthesia with intraperitoneally-injected 2.5% avertin. The animals were perfused with 3% paraformaldehyde with 0.6% glutaraldehyde in phosphate-buffered saline (PBS). Brains were left in the skulls for 24 hrs in 3% paraformaldehyde, then removed and stored in PBS. After weighing, the brains were transferred to a 30% sucrose solution containing 0.08% sodium azide in PBS. They were then frozen on dry ice, mounted with Tissue-TEK O.C.T. compound (Sakura, Torrance, CA), and sliced coronally into 25 μm sections on a cryostat (Microm HM 500M, Richard-Allan Scientific, Calamazoo, MI). The sections are collected and stored in PBS with 0.08% sodium azide at 4 degrees.

Neuropathological analysis

A series of 25 μm-thick coronal sections spaced 200 μm apart spanning the striatum were stained with NeuN antibody (1:100; Chemicon, Temecula, CA) overnight at room temperature, followed by incubation with biotinylated anti-mouse antibody (1:200; Vector Laboratories, Burlingame,

CA). The signal was amplified with an ABC Elite kit (Vector) and detected with diaminobenzidine (DAB; Pierce, Rockford, IL). Striatal volume was determined from a series of mounted sections using StereoInvestigator software (Microbrightfield, Williston, VT). Briefly, striatal volumes were determined by tracing the perimeter of the striatum in serial sections spanning the striatum.

Statistical analysis

Data are expressed as means±SEM. Whenever suitable, results were interpreted using one-way ANOVA with a Student-Newman-Keuls (SNK) post-hoc test. Pairwise comparisons between genotypes/treatments at individual time points were assessed with a Student's t-test. Differences were considered statistically significant when $P < 0.05$.

5.3. Results

Acute arimoclomol treatment does not protect against quinolinic acid-mediated excitotoxicity in vivo

To examine whether acute treatment with arimoclomol results in protection in the presence of a stressor, namely the NMDA receptor agonist quinolinic acid, 3-months old FVB/N animals were pretreated with 40 mg/kg of arimoclomol i.p. followed by an intrastriatal injection of 25 nmol QA 30 min later. Dying neurons following intrastriatal injection of QA were detected using FluoroJade, a fluorescent marker of degenerating neurons. Analysis revealed no significant difference in QA-induced lesion volume in animals pretreated with arimoclomol compared to controls (P=0.351) (Figure 5.1A).

Acute treatment with arimoclomol fails to up-regulate HSP-70 and HSP-90 expression

To assess whether acute treatment with arimoclomol results in up-regulation of brain HSP-70 and HSP-90 proteins, 3 months old FVB/N animals were treated with 40 mg/kg of arimoclomol i.p. and whole brains were collected 0.5, 1, 3, and 6 hrs post-injection. Immunoblot analysis revealed no up-regulation of HSP-70 or HSP-90 in arimoclomol-treated animals compared to vehicle-treated (t=0') animals at any of the time points assessed (Figure 5.1B).

Short-term arimoclomol treatment does not protect against quinolinic acid-mediated excitotoxicity in vivo

To assess whether short-term treatment with high-dose arimoclomol in the presence of a physiological stressor (e.g. polyglutamine expansion in the *HD* gene) is necessary to confer protection against quinolinic acid-mediated striatal lesions, 3-months old YAC128 animals were treated with 200 mg/kg arimoclomol for 7 days followed by intrastriatal injection of 25 nmol QA on day 7. After 7 additional days of treatment with arimoclomol, animals were sacrificed and lesion volume as assessed with Fluorojade-staining was quantified (Figure 5.2A). Analysis

revealed no significant difference in lesion volume in arimoclomol-treated YAC128 animals compared to untreated YAC128 controls (P=0.604) (Figure 5.2B).

Long-term treatment with arimoclomol does not lead to improved motor function in the YAC128 HD animals

To evaluate the effects of long-term treatment with arimoclomol on the motor function, YAC128 HD animals and WT littermates were treated with arimoclomol for 10-months starting at 2 months of age, and performance on the accelerating and fixed-speed rotarod tests of motor function at 2 (baseline), 4, 6, 8, 10, and 12 months of age was assessed. Data analysis revealed no significant effect of arimoclomol treatment on motor function in WT animals as there was no significant difference between arimoclomol- and vehicle-treated WT animals at 4, 6, 8, and 12 months of age on the accelerating rotarod and no significant difference between arimoclomol- and vehicle-treated WT animals on the fixed-speed rotarod at any of the timepoints evaluated (Figure 5.3A). On the accelerating rotarod task, untreated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months of age compared to WT. Arimoclomol-treated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months compared to WT. Furthermore, arimoclomol-treated YAC128 animals were not statistically different from untreated YAC128 animals at any of the measured time points. On the fixed-speed rotarod task, untreated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months of age compared to WT. Arimoclomol-treated YAC128 animals had a significantly lower performance at 6, 8, 10, and 12 months compared to WT. Furthermore, arimoclomol-treated YAC128 animals were not statistically different from untreated YAC128 animals at any of the measured time points (Figure 5.3B).

Long-term treatment with arimoclomol fails to rescue striatal neuropathology in the YAC128 HD animals

The effect of long-term arimoclomol treatment on neuropathology in the YAC128 HD animals was assessed. The brain weights of arimoclomol-treated WT animals were not statistically different from those of untreated WT (P=0.175). Untreated YAC128 animals had significantly lower brain weight compared to untreated WT animals (P=0.0017). Similarly, arimoclomol-treated YAC128 animals had significantly lower brain weights compared to untreated WT (P=0.0257). Furthermore, the brain weights of untreated and arimoclomol-treated YAC128 animals were not statistically different (P=0.090) (Figure 5.4A). The striatal volume of untreated YAC128 animals was significantly lower than that of untreated WT animals at 12 months of age (P=0.029). Further, the striatal volume of treated WT animals was significantly lower than that of untreated WT animals (P=0.024). The striatal volume of treated YAC128 animals was significantly reduced compared to that of untreated WT animals (P=0.015), but was not statistically different from that of untreated YAC128 animals (P=0.463) (Figure 5.4B).

5.4. Discussion

The aim of this study was to evaluate whether induction of HSPs by treatment with arimoclomol, a co-inducer of the transcriptional factor Hsf-1, would lead to improvements in the acute lesional QA model of HD or the transgenic YAC128 HD mouse model. While some studies suggested little or no effects of over-expression of specific HSPs, such as HSP-70 and HSP-27, on the HD phenotype in transgenic HD mouse models (Hansson et al., 2003; Hay et al., 2004; Zourlidou et al., 2007), others have reported significant improvements in cellular (Jana et al., 2000), fly (McLear et al., 2008), and mouse models of HD (Perrin et al., 2007; Vacher et al., 2005). Furthermore, suppression of HSP-70 expression has been linked to enhanced vulnerability to polyglutamine-mediated toxicity in cellular models of HD (King et al., 2008; Tagawa et al., 2007). We, therefore, hypothesized that up-regulation of multiple HSPs by prolonging the activation of Hsf-1 using a pharmacological intervention may provide added benefit in the YAC128 HD animals.

We report here that acute treatment with arimoclomol (80 mg/kg) failed to up-regulate HSP-70 or HSP-90 expression in the brains of FVB/N animals. This lack of up-regulation of HSP-70 and HSP-90 may reflect an inadequate availability of arimoclomol at an appropriate level in the brain, or an insufficient amount of time for arimoclomol to act on its target. Alternatively, as arimoclomol acts as a co-inducer of HSP expression by stabilizing an activated Hsf-1 as part of an initiated stress response (Kalmar et al., 2008; Kieran et al., 2004), the lack of up-regulation of HSPs may reflect the absence of stress conditions in the naïve FVB/N animals.

Expansion of polyglutamine stretches into the pathogenic range in proteins associated with neurodegenerative disorders represents a cellular stress condition that leads to the degeneration

and loss of vulnerable neuronal populations over time. In order to examine whether treatment with arimoclomol can up-regulate HSPs in the presence of expanded polyglutamine-mediated cellular stress, YAC128, which express human htt with an expanded polyglutamine tract, were treated for 7 days with arimoclomol. A dose of arimoclomol five fold higher (i.e. 200 mg/kg) than that used in the single acute treatment study was administered to ensure sufficiently high levels of the drug are available to effect its action. Following intrastriatal injection of QA, which represents an additional acute stress, the animals were treated for an additional 7 days before being sacrificed for analysis. Analysis of striatal lesion volume showed no protective effect of arimoclomol treatment compared to control. The length of time of treatment (14 days in total) as well as the high dose of arimoclomol employed suggest that the lack of efficacy is unlikely to reflect inadequate availability in the brain or insufficient time for arimoclomol to act on its target. The use of YAC128 animals further suggests that treatment with arimoclomol may not result in HSPs induction in the presence of polyglutamine-mediated cellular stress or that any arimoclomol-mediated induction of HSPs in YAC128 animals is ineffective in protecting against QA-mediated striatal lesioning. Alternatively, the striatal lesioning created by intrastriatal QA administration may represent a supraphysiological stress condition that may be intractable to treatment with arimoclomol and incompatible with its mode of action.

To assess the potential therapeutic value of arimoclomol treatment, we treated YAC128 animals starting at 2 months of age with 80 mg/kg/day of arimoclomol. Our findings demonstrates that treatment with arimoclomol fails to improve the motor function of YAC128 animals and wildtype controls as assessed by the accelerating and fixed-speed rotarod tasks. We further demonstrate that treatment with arimoclomol fails to improve the neuropathology of YAC128 animals as assessed by brain weight and striatal volume. As the dose employed in this study was

four times that found to be efficacious in a mouse model of ALS, and given the length of time in which the animals received the treatment (10 months), the lack of efficacy is unlikely to reflect inadequate dosing or availability. The results instead likely suggest that treatment with arimoclomol is ineffective in improving the motor and neuropathological phenotype in the YAC128 animals.

The results of this study also support the notion that one cannot necessarily extrapolate from one animal model of disease to another: that arimoclomol was able to up-regulate the heat shock response in a mouse model of ALS leading to improved phenotype does not necessarily mean that similar effects on heat shock protein induction and phenotype improvement would be observed in a mouse model of HD.

Furthermore, the findings argue in favor of using gating experiments to guide decisions about whether or not to move ahead with or continue initiated long-term efficacy trials in animals. That no effect of arimoclomol treatment was observed on QA-mediated striatal lesions, a surrogate of the excitotoxicity phenomenon being targeted, may be taken as an argument against the initiation of a long-term efficacy trial of arimoclomol in the YAC128 mice, or at least early termination of the on-going study. Incorporation of such gating experiments should benefit future trials, and may provide considerable savings in both time and cost, and allow for resources to be shifted to more promising approaches.

As we were unable to demonstrate an induction of HSPs following treatment with arimoclomol in the different experimental paradigms employed, our findings do not rule out the potential value of induction of a heat shock protein response as a therapy in HD. Indeed, the promise held by such an approach as a therapy has been demonstrated in several experimental models of

polyglutamine-mediated neurodegeneration (Fujikake et al., 2008; Fujimoto et al., 2005; Katsuno et al., 2005; Zhang and Sarge, 2007) and warrants further investigation.

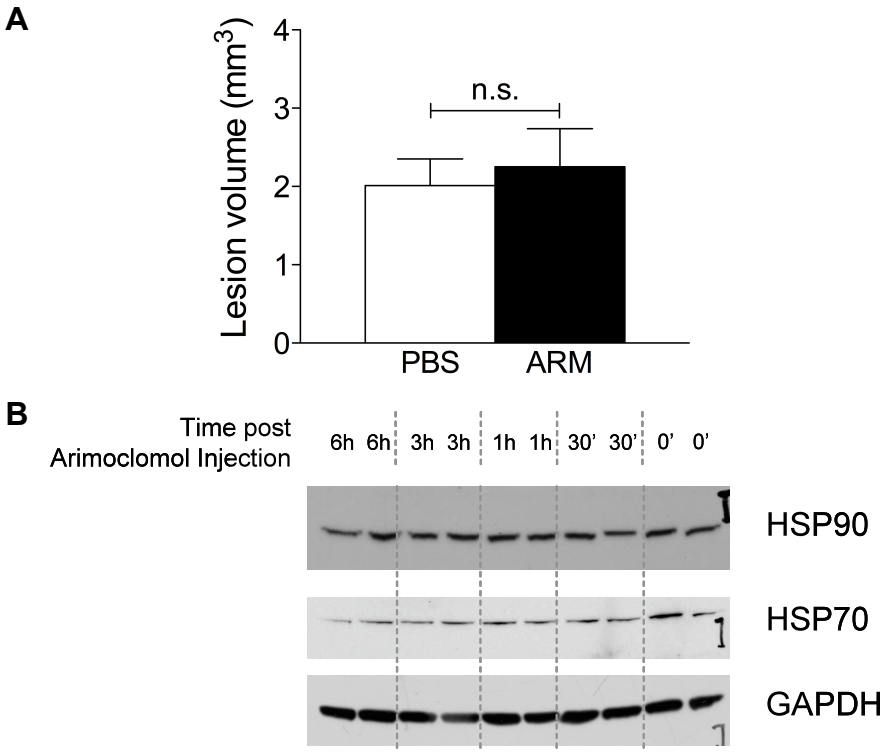


Figure 5.1 Acute arimoclomol treatment fails to upregulate HSP-70 and HSP-90 expression, and does not protect against quinolinic acid-mediated excitotoxicity in vivo

(A) To examine whether acute treatment with arimoclomol results in protection against quinolinic acid-mediated striatal lesions, 3-months old FVB/N animals were pretreated with 40 mg/kg of arimoclomol i.p. followed by an intrastriatal injection of 25 nmol QA 30 min later. Dying neurons following intrastriatal injection of QA were detected using FluoroJade, a fluorescent marker of degenerating neurons. Analysis revealed no significant difference in lesion volume in arimoclomol-pretreated animals compared to controls (P=0.351). (B) To assess whether acute treatment with arimoclomol results in upregulation of brain HSP-70 and HSP-90 proteins, 3 months old animals were treated with 40 mg/kg of arimoclomol i.p. and brains were collected 0.5, 1, 3, and 6 hrs post-injection. Immunoblot analysis showed no upregulation of HSP-70 or HSP-90 in arimoclomol-treated animals compared to vehicle-treated (t=0') animals at any of the time points assessed. Data are represented as means±SEM; n=7 (WT), and 8 (YAC128). n.s.=no significant difference.

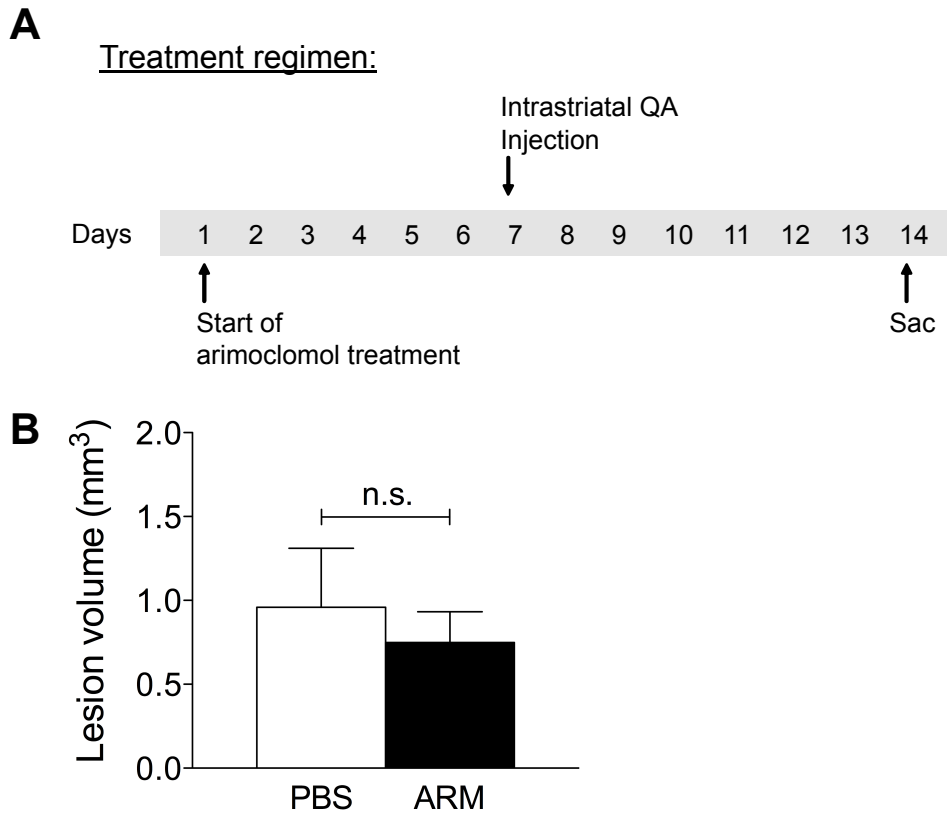


Figure 5.2 Short-term arimoclomol treatment does not protect against quinolinic acid-mediated excitotoxicity in vivo

(A) To assess whether short-term treatment with arimoclomol results in protection against quinolinic acid-mediated striatal lesions in the presence of the HD mutation, 3-months old YAC128 animals were treated with 200 mg/kg arimoclomol for 7 days followed by intrastriatal injection of 25 nmol QA on day 7. After 7 additional days of treatment with arimoclomol, animals were sacrificed and lesion volume as assessed with Fluorojade-staining was quantified. (B) Analysis revealed no significant difference in lesion volume in arimoclomol-treated YAC128 animals compared to untreated YAC128 controls (P=0.604). Data are represented as means±SEM; n=6 (PBS), and 6 (ARM). n.s.=no significant difference.

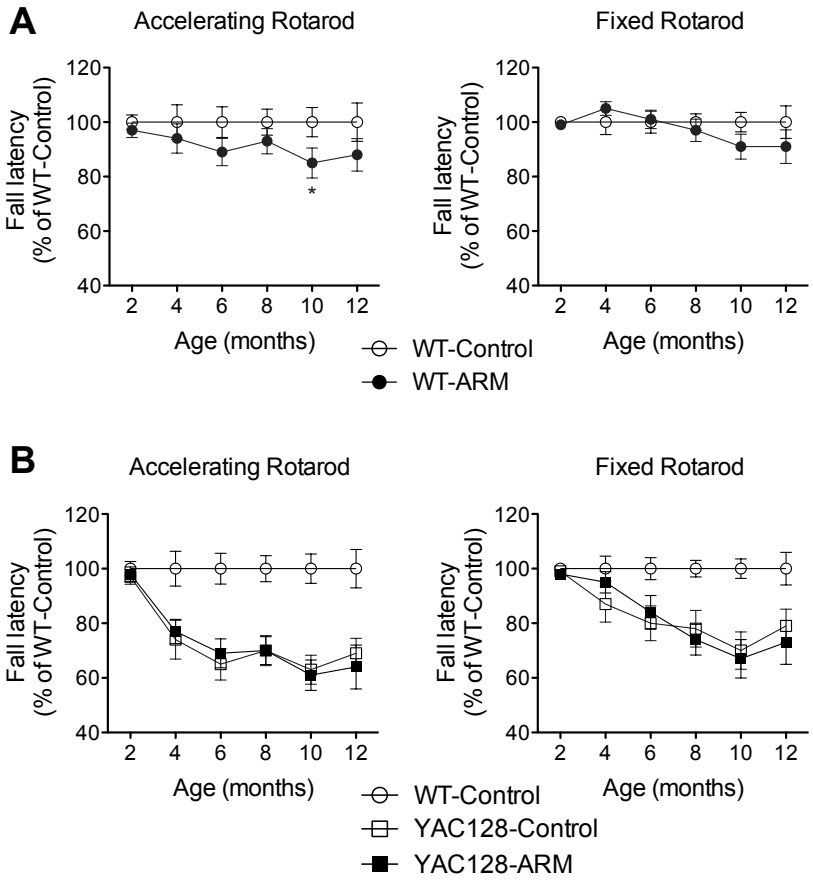


Figure 5.3 Long-term treatment with arimoclomol does not lead to improved motor function in the YAC128 HD animals

To evaluate the effects of long-term treatment with arimoclomol on the motor function, YAC128 HD animals and WT littermates were treated with arimoclomol for 10-months starting at 2 months of age, and performance on the accelerating and fixed-speed rotarod tests of motor function at 2 (baseline), 4, 6, 8, 10, and 10 months of age was assessed. (A) Data analysis revealed no significant effect of arimoclomol treatment on motor function in WT animals as there was no significant difference between arimoclomol- and vehicle-treated WT animals at 4, 6, 8, and 12 months of age on the accelerating rotarod and no significant difference between arimoclomol- and vehicle-treated WT animals on the fixed-speed rotarod at any of the timepoints evaluated. (B) On the accelerating rotarod task, untreated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months of age compared to WT. Arimoclomol-treated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months compared to WT. Furthermore, arimoclomol-treated YAC128 animals were not statistically different from untreated YAC128 animals at any of the measured time points. On the fixed-speed rotarod task, untreated YAC128 animals had a significantly lower performance at 4, 6, 8, 10, and 12 months of age compared to WT. Arimoclomol-treated YAC128 animals had a significantly lower performance at 6, 8, 10, and 12 months compared to WT. Furthermore, arimoclomol-treated YAC128 animals were not statistically different from untreated YAC128 animals at any of the measured time points. Data are presented as mean±SEM. *P<0.05 compared to untreated WT animals.

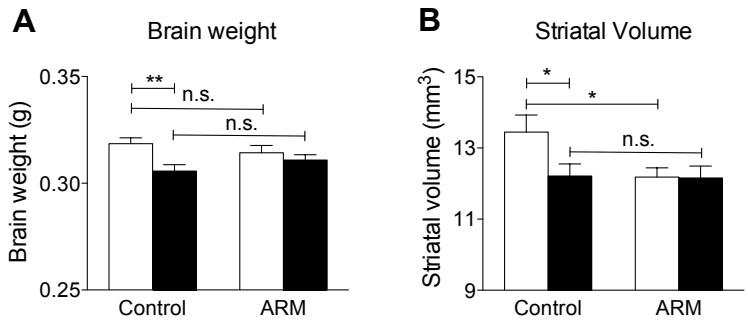


Figure 5.4 Long-term treatment with arimoclomol treatment fails to rescue striatal neuropathology in the YAC128 HD animals

The effect of long-term arimoclomol treatment on neuropathology in the YAC128 HD animals was assessed. (A) The brain weights of arimoclomol-treated WT animals were not statistically different from those of untreated WT (P=0.175). Untreated YAC128 animals had significantly lower brain weight compared to untreated WT animals (P=0.0017). Similarly, arimoclomol-treated YAC128 animals had significantly lower brain weights compared to untreated WT (P=0.0257). Furthermore, the brain weights of untreated and arimoclomol-treated YAC128 animals were not statistically significant (P=0.090). (B) The striatal volume of untreated YAC128 animals was significantly lower than that of untreated WT animals at 12 months of age (P=0.029). Further, the striatal volume of treated WT animals was significantly lower than that of untreated WT animals (P=0.024). The striatal volume of treated YAC128 animals significantly reduced compared to that of untreated WT animals (P=0.015), but was not statistically different from that of untreated YAC128 animals (P=0.463). Data are presented as mean±SEM. *P<0.05; **P<0.001; n.s.=no significant difference.

5.5. References

Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J., Martin, J.B., 1986. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 321**,** 168-71.

Beal, M.F., Kowall, N.W., Swartz, K.J., Ferrante, R.J., Martin, J.B., 1988. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J Neurosci. 8**,** 3901-8.

Beal, M.F., Ferrante, R.J., Swartz, K.J., Kowall, N.W., 1991. Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. J Neurosci. 11**,** 1649-59.

Cudkowicz, M.E., Shefner, J.M., Simpson, E., Grasso, D., Yu, H., Zhang, H., Shui, A., Schoenfeld, D., Brown, R.H., Wieland, S., Barber, J.R., Consortium, N.A., 2008. Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. Muscle Nerve. 38**,** 837-44.

Dedeoglu, A., Ferrante, R.J., Andreassen, O.A., Dillmann, W.H., Beal, M.F., 2002. Mice overexpressing 70-kDa heat shock protein show increased resistance to malonate and 3-nitropropionic acid. Experimental Neurology. 176**,** 262-5.

Fan, M.M.Y., Raymond, L.A., 2007. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. Prog Neurobiol. 81**,** 272-93.

Ferrante, R.J., Kowall, N.W., Beal, M.F., Richardson, E.P., Bird, E.D., Martin, J.B., 1985. Selective sparing of a class of striatal neurons in Huntington's disease. Science. 230**,** 561-3.

Fujikake, N., Nagai, Y., Popiel, H.A., Okamoto, Y., Yamaguchi, M., Toda, T., 2008. Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem. 283**,** 26188-97.

Fujimoto, M., Takaki, E., Hayashi, T., Kitaura, Y., Tanaka, Y., Inouye, S., Nakai, A., 2005. Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. J Biol Chem. 280**,** 34908-16.

Furtado, J.C., Mazurek, M.F., 1996. Behavioral characterization of quinolinate-induced lesions of the medial striatum: relevance for Huntington's disease. Experimental Neurology. 138**,** 158-68.

Hansson, O., Nylandsted, J., Castilho, R.F., Leist, M., Jäättelä, M., Brundin, P., 2003. Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. Brain Res. 970**,** 47-57.

Hargitai, J., Lewis, H., Boros, I., Rácz, T., Fiser, A., Kurucz, I., Benjamin, I., Vígh, L., Pénzes, Z., Csermely, P., Latchman, D.S., 2003. Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. Biochem Biophys Res Commun. 307**,** 689-95.

Hay, D.G., Sathasivam, K., Tobaben, S., Stahl, B., Marber, M., Mestril, R., Mahal, A., Smith, D.L., Woodman, B., Bates, G.P., 2004. Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. Hum Mol Genet. 13**,** 1389-405.

Jana, N.R., Tanaka, M., Wang, G.h., Nukina, N., 2000. Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum Mol Genet. 9**,** 2009-18.

Kalmar, B., Novoselov, S., Gray, A., Cheetham, M.E., Margulis, B., Greensmith, L., 2008. Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. J Neurochem. 107**,** 339-50.

Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., Doyu, M., Sobue, G., 2005. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci USA. 102**,** 16801-6.

Kieran, D., Kalmar, B., Dick, J.R.T., Riddoch-Contreras, J., Burnstock, G., Greensmith, L., 2004. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med. 10**,** 402-5.

King, M.A., Goemans, C.G., Hafiz, F., Prehn, J.H.M., Wyttenbach, A., Tolkovsky, A.M., 2008. Cytoplasmic inclusions of Htt exon1 containing an expanded polyglutamine tract suppress execution of apoptosis in sympathetic neurons. J Neurosci. 28**,** 14401-15.

Landwehrmeyer, G.B., Standaert, D.G., Testa, C.M., Penney, J.B., Young, A.B., 1995. NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. J Neurosci. 15**,** 5297-307.

McLear, J.A., Lebrecht, D., Messer, A., Wolfgang, W.J., 2008. Combinational approach of intrabody with enhanced Hsp70 expression addresses multiple pathologies in a fly model of Huntington's disease. FASEB J. 22**,** 2003-11.

Ohtsuka, K., Suzuki, T., 2000. Roles of molecular chaperones in the nervous system. Brain Res Bull. 53**,** 141-6.

Perrin, V., Régulier, E., Abbas-Terki, T., Hassig, R., Brouillet, E., Aebischer, P., Luthi-Carter, R., Déglon, N., 2007. Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. Mol Ther. 15**,** 903-11.

Popoli, P., Pèzzola, A., Domenici, M.R., Sagratella, S., Diana, G., Caporali, M.G., Bronzetti, E., Vega, J., Scotti de Carolis, A., 1994. Behavioral and electrophysiological correlates of the quinolinic acid rat model of Huntington's disease in rats. Brain Res Bull. 35**,** 329-35.

Pouladi, M., Bezprozvanny, I., Raymond, L.A., Hayden, M., 2006. Molecular Pathogenesis of Huntington's Disease: The Role of Excitotoxicity. Genetic Instabilities and Neurological Diseases.

R. Hayden, M., 1981. Huntington's Chorea.

Shear, D.A., Dong, J., Gundy, C.D., Haik-Creguer, K.L., Dunbar, G.L., 1998. Comparison of intrastriatal injections of quinolinic acid and 3-nitropropionic acid for use in animal models of Huntington's disease. Prog Neuropsychopharmacol Biol Psychiatry. 22**,** 1217-40.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12**,** 1555-67.

Tagawa, K., Marubuchi, S., Qi, M.-L., Enokido, Y., Tamura, T., Inagaki, R., Murata, M., Kanazawa, I., Wanker, E.E., Okazawa, H., 2007. The induction levels of heat shock protein 70 differentiate the vulnerabilities to mutant huntingtin among neuronal subtypes. J Neurosci. 27**,** 868-80.

Vacher, C., Garcia-Oroz, L., Rubinsztein, D.C., 2005. Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. Hum Mol Genet. 14**,** 3425-33.

Young, A.B., Greenamyre, J.T., Hollingsworth, Z., Albin, R., D'Amato, C., Shoulson, I., Penney, J.B., 1988. NMDA receptor losses in putamen from patients with Huntington's disease. Science. 241**,** 981-3.

Zhang, Y.-Q., Sarge, K.D., 2007. Celastrol inhibits polyglutamine aggregation and toxicity though induction of the heat shock response. J Mol Med. 85**,** 1421-8.

Zourlidou, A., Gidalevitz, T., Kristiansen, M., Landles, C., Woodman, B., Wells, D.J., Latchman, D.S., de Belleroche, J., Tabrizi, S.J., Morimoto, R.I., Bates, G.P., 2007. Hsp27 overexpression in the R6/2 mouse model of Huntington's disease: chronic neurodegeneration does not induce Hsp27 activation. Hum Mol Genet. 16**,** 1078-90.

6. Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin[5]

6.1. Introduction

Huntington disease is an incurable autosomal dominant neurological disorder characterized by a triad of motor, cognitive, and affective disturbances (Hayden, 1981). The disease is caused by a trinucleotide CAG expansion in exon 1 of 67 axons comprising the *HD* gene leading to an extended polyglutamine tract in the huntingtin protein. The cardinal and early neuropathological feature of HD is atrophy of the caudate nucleus and the putamen (the neostriatum), with selective loss of medium-sized spiny neurons within the striatum.

Although onset of HD is clinically determined on the basis of motor performance, prominent affective abnormalities, particularly depression, are common features of the disease. Depression has been reported to occur in as many as 40% to 50% of HD patients and may predate onset of motor symptoms by more than 10 years (Duff et al., 2007; Folstein and Folstein, 1983; Heathfield, 1967; Kirkwood et al., 2001; Pflanz et al., 1991). Indeed, the prevalence of depressive symptoms has been demonstrated to be increased in presymptomatic mutation carries compared to non-mutation carriers (Julien et al., 2007; Marshall et al., 2007).

The etiology of depression in HD is thought to be multifactorial with both psychosocial and neurobiological contributions. The realization of the intransigent nature of this fatal illness, along with increased functional disability and loss of capacity to carry out daily functions are considered to be important precipitating factors. Also, social upheaval in families with HD with

[5] A version of this chapter has been published. Pouladi MA, Graham RK, Karasinska JM, Xie Y, Santos RD, Petersén A, Hayden MR. Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. Brain (2009) vol. 132 (Pt 4) pp. 919-32

significant discord and stress may also contribute. There is evidence however that biological factors do contribute independently to depression in HD. Indeed, depression in HD often antedates motor and cognitive impairments by many years (Duff et al., 2007; Folstein and Folstein, 1983; Pflanz et al., 1991). Further, depression is often observed in other disorders of the basal ganglia such as Parkinson disease and corticobasal degeneration, where it may also predate other symptoms (Lieberman, 2006; Litvan et al., 1998), implicating disruptions of neural circuits involving the basal ganglia as the precipitating factor leading to depression in these disorders.

As a first step towards unraveling the pathogenic mechanisms underlying the affective disturbances in HD, we examined whether the depression state observed in patients is modeled in the transgenic YAC HD animals. The YAC HD animals express the entire human HD gene under the control of the endogenous huntingtin promoter and regulatory elements and recapitulate many features of the human condition, including motor and cognitive, and selective neuronal deficits (Hodgson et al., 1999; Slow et al., 2003; Van Raamsdonk et al., 2005b). The Porsolt forced swim test (FST) and a sucrose intake test were used to assess depressive behaviour in the YAC HD animals compared to their littermates in the same environment. The Porsolt FST is one of the most widely employed paradigms for assessing 'depression' phenotypes and antidepressant action in rodents (Cryan et al., 2002; Porsolt et al., 1977a; Porsolt et al., 1977b). The test model is based on the observation that a rodent, when forced to swim in a restricted space where there is no possibility for escape, will cease to struggle after an initial period of activity and simply float. Increased immobility is interpreted as a depressive state, representing signs of psychomotor retardation and/or despair (Cryan et al., 2002; Porsolt et al., 1977a; Porsolt et al., 1977b). The sucrose intake test assesses one of the major symptoms of depression, anhedonia (inability to experience pleasure). Reduced sucrose consumption and

preference is interpreted as a decreased sensitivity to reward (Willner et al., 1987). Our results indicate that early depressive phenotypes are indeed present in the YAC HD animals, independent of disease duration and CAG repeat length, and closely mirror the symptoms observed in patients with HD.

6.2. Materials and methods

Animals

The experiments were performed on wildtype animals and transgenic YAC animals expressing the entire human *HD* gene under the control of the endogenous huntingtin promoter and regulatory elements on the FVB/N strain background. YAC18 animals express human wildtype huntingtin with 18 CAG repeats (Hodgson et al., 1996). YAC46, YAC72, and YAC128 express human mutant huntingtin with 46, 72, and 120 CAG repeats, respectively (Hodgson et al., 1999; Slow et al., 2003). The most characterized of these is the YAC128 line which recapitulates several features of human HD including progressive cognitive deficits starting at 2 months of age, motor dysfunction by 3 months of age, followed by selective neuropathology with striatal atrophy clearly evident by 8 months of age (Slow et al., 2003; Van Raamsdonk et al., 2005b; Van Raamsdonk et al., 2005c; Lerch et al., 2007). C6R YAC transgenic animals express human mutant huntingtin with 133 CAG repeats that has been mutated at aa 586 to prevent cleavage by caspase-6 (Graham et al., 2006). All animals were bred at the animal facility of the Centre for Molecular Medicine and Therapeutics at the University of British Columbia and were group-housed in numerical birth order with littermates of mixed genotype. Mice were maintained on a 12 light : 12 dark light cycle (lights on at 2300) and behavioural testing was conducted during the dark phase. Experimenters were blind to the genotype of the mice. Food and water was provided *ad libitum*. Unless stated otherwise, experiments were performed on 3- to 4-month-old and 8- to 9-month-old mice and their non-transgenic littermates. Experiments were performed with the approval of the animal care committee at the University of British Columbia.

Forced swim test procedure and scoring

Immobility in the FST is a commonly-used measure of depression in rodents (Cryan et al., 2002; Porsolt et al., 1977a; Porsolt et al., 1977b). The FST was conducted by placing mice in individual cylinders (25cm-tallx19cm-wide) filled with room temperature water (23-25°C) to a depth of 15cm for a period of 6 minutes. The test sessions were recorded by a video camera placed directly above the cylinders. The sessions were examined blind and the last 4 minutes of the test session was scored using a time-sampling technique to rate the predominant behaviour over 5-second intervals. The following behaviours were measured and recorded at the end of every 5 seconds: swimming, immobility, and climbing. Independent cohorts of animals were used throughout the study, and no repeat testing was performed. The number of animals tested for each genotype and time-point is stated in the figure legend. As no significant difference was observed in climbing behaviour between the genotypes in any of the comparisons made throughout the study, climbing behaviour data will not be shown for the sake of clarity.

Simple test of swimming ability

To assess whether the ability to swim in YAC128 HD animals is impaired, animals were tested in a simple swimming test. In this test, mice were placed at one end of a linear swimming chamber (76 x 13 cm; water depth, 9 cm; platform, 6 x 13 cm) and trained to reach a platform at the other end of the chamber in the shortest amount of time to escape from the water. Mice were trained with three consecutive sessions 5 min apart. Mice were tested with seven successive trials and swimming speed for each mouse was recorded for each trial.

Test of motor function

Training for the test of motor function was carried out at 2 months of age. Mice were trained for three days with three trials per day on a fixed-speed rotarod (UGO Basile, Comerio, Italy).

Training sessions ran for 120 sec at 18 revolutions per minute (RPM) and were spaced 1 hr apart. Testing took place during the dark cycle and was carried out at 3 and 12 months of age. Motor co-ordination and balance were assessed using the accelerating rotarod task. In this test, the rotarod accelerated from 5 to 40 RPM over 5 minutes and performance was assessed by the amount of time that a mouse could remain running on the rotarod; the maximum score is 300 seconds. Rotarod scores are the average of three trials spaced 2 hr apart.

Sucrose consumption measurement

A modified sucrose consumption test based on that described by Strekalova et al (Strekalova et al., 2004) was performed. Briefly, individually-housed animals were given ad libitum access to food and two-bottles of water. On the day preceding intake measurements, one of the water bottles was replaced with a bottle containing 2% sucrose solution. After 24 hr, the amount of sucrose solution and water intake by each animal was estimated by weighing the bottles. The sucrose preference was calculated as the ratio of the amount of 2% sucrose solution consumed to the total amount of solution consumed ([2% sucrose solution] ÷ [2% sucrose solution + water]) normalized to kg body weight.

Stress-induced hyperthermia test procedure

The procedure for the stress-induced hyperthermia (SIH) test of anxiety was adapted from van der Heyden et al. (Van der Heyden et al., 1997). Measurement of the basal temperature in mice with a rectal probe represents a stressor that causes an increase in the rectal temperature and that can be inhibited by anxiolytics (Zethof et al., 1994). The rectal temperature of individually-housed mice was measured twice in each mouse to the nearest 0.1°C: at t=0 min (T1) and t=+10 min (T2). The SIH was calculated as the difference $\Delta T = T2-T1$. The number of animals tested is n=18 for WT, 20 for YAC128.

Anti-depressant treatment

Mice were treated with two commonly used anti-depressants, fluoxetine, a selective serotonin reuptake inhibitor, and imipramine, a tricyclic antidepressant.

Animals were treated with fluoxetine hydrochloride (Sigma, St. Louis, MO, USA) at 4 months of age, daily for 21 days. Fluoxetine hydrochloride was dissolved in 0.9% saline and administered intraperitoneally (i.p.) at a dose of 20mg/kg in a volume of 10mL/kg. Control animals received 0.9% saline in a volume of 10mL/kg, i.p (Duncan et al., 1996). The number of animals tested is n=9 per treatment.

Animals were treated with imipramine (Sigma, St. Louis, MO, USA) daily for 21 days. Imipramine was dissolved in 0.9% saline and administered i.p. at a dose of 10mg/kg b.i.d. in a volume of 10mL/kg. Control animals received 0.9% saline in a volume of 10mL/kg, i.p. b.i.d (Przegalinski et al., 1995). The number of animals tested is n=5 per treatment.

Statistical analysis

Data are expressed as means±SEM. Whenever suitable, results were interpreted using one-way ANOVA with a Tukey test. Pair-wise comparisons between genotypes or treatments at individual time points were assessed with a Student's post-hoc test. Linear regression analyses for r^2 and P values were performed with GraphPad Prism version 4.02. Differences were considered statistically significant when $P < 0.05$.

6.3. Results

YAC128 HD animals display depressive behaviour

To determine whether YAC128 exhibit depressive behaviour, 3 months old YAC128, YAC18, and wildtype animals were subjected to the FST. YAC128 animals spent a significantly longer time in an immobile state compared to YAC18 (P=0.027) and wildtype animals (P=0.008) (Figure 6.1). Consistent with the increased immobility displayed, YAC128 animals spent significantly less time swimming compared to YAC18 (P<0.001) and wildtype animals (P=0.002). No significant differences in immobility or swimming behaviours between YAC18 and wildtype animals were observed (P=0.986, and P=0.733, respectively). (Figure 6.1).

The severity of the depressive behaviour does not increase over time in the YAC128 HD animals

The severity of depressive symptoms in HD patients has been shown not to correlate with the duration of disease (Craufurd et al., 2001). To examine whether the severity of depressive behaviours in YAC128 animals worsened with time, the performance of YAC128 animals and wildtype littermates in the FST at 3, 8, and 12 months of age were compared. As seen at 3 months of age, YAC128 animals spent a significantly longer time in an immobile state compared to their wildtype littermates (P=0.002 and 0.0187 for 8 and 12 months old mice, respectively) (Figure 6.2A) and significantly less time swimming (P=0.001 and 0.0295 for 8 and 12 months old mice, respectively) (Figure 6.2B). Consistent with the findings in HD patients, no significant differences were observed in depressive behavior as signified by immobility between 3, 8, and 12 months old YAC128 animals (P=0.965) or swimming times (P=0.885) (Figure 6.2A and B). Similarly, no significant differences were noted in immobility or swimming times between 3, 8, and 12 months old WT animals (P=0.218, and P=0.267, respectively) (Figure 6.2A and B).

These results are in contrast to the progressive nature of motor and neuropathological features in these mice.

The depressive behaviour is observed to the same extent in male and female animals

To examine whether a gender difference exists in the performance of animals in the forced swim test, immobility time of males was compared to that of females at 8 months of age. Similar to female YAC128 animals, male YAC128 animals spent a significantly longer time in an immobile state compared to male WT littermates (96±21 sec for YAC128 and 24±9 sec for WT, n=10 for YAC128 and 8 for WT, P=0.011). However, no significant difference in immobility between male and female YAC128 animals were observed (96±21 sec for males and 90±21 sec for females, n=10 for males and 9 for females, P= 0.844). Similarly, no significant difference in immobility between male and female WT animals were observed (24±9 sec for males and 11±5 sec for females, n=8 for males and 9 for females, P= 0.228). As no gender differences were observed, all the subsequent cohorts of animals employed in this study are of mixed gender.

The extent of the depressive behaviour is independent of animal body weight

The body weight of 3 months old YAC128 animals does not differ from that of wildtype littermates (P=0.135), while increased body weight is observed in 8 months old (P<0.001) and 12 months old YAC128 animals (P<0.01) compared to WT littermates (Figure 6.2C). To examine whether increased body weight may contribute to immobility in the forced swim test, immobility time of 3, 8, and 12-months old animals was plotted against body weight. Regression analysis revealed no correlation between body weight and immobility time (r^2=0.0317, P=0.159) (Figure 6.2D).

The ability to swim is not impaired in the YAC128 HD animals despite motor dysfunction

To assess whether the ability to swim in YAC128 HD animals is impaired, animals were tested in a simple swimming test. In this test, mice were placed at one end of a linear swimming chamber and trained to reach a platform at the other end of the chamber to escape from the water. Mice were tested on seven successive trials and the swimming speed (distance traveled per second) for each mouse was assessed for each trial. There was no significant difference in the swimming speed of YAC128 animals compared to WT animals at either 3 or 12 months of age in any of the trials (Figure 6.3A). Further, the average swimming speed over the 7 trials was not significantly different between YAC128 and WT animals at either 3 or 12 months of age (Figure 6.3B), despite significantly lower performance in the rotarod test of motor function by YAC128 animals compared to WT animals at these time points (Figure 6.3C).

The severity of the depressive behaviour in YAC transgenic HD animals is independent of CAG repeat length

The severity of depressive symptoms in HD patients has been shown to be independent of CAG length (Berrios et al., 2001; Craufurd et al., 2001; Zappacosta et al., 1996). To assess whether the severity of the depressive behaviour correlated with CAG repeat length, wildtype, YAC46, YAC72, and YAC128 animals were subjected to the FST. Similar to YAC128 animals, YAC46 and YAC72 animals were immobile for significantly longer times than wildtype animals (vs. wildtype: P=0.026 for YAC46, <0.001 for YAC72, and <0.001 for YAC128) (Figure 6.4). No difference in the extent of immobility between YAC46, YAC72, and YAC128 animals was observed (YAC46 vs. YAC72 and YAC128: P=0.054 and 0.828, respectively; YAC72 vs. YAC128: P=0.125) (Figure 6.4). YAC46, YAC72, and YAC128 animals also spent less time swimming compared to wildtype animals, although the difference did not reach statistical

significance in the case of the YAC46 animals (vs. wildtype: P=0.067 for YAC46, P<0.001 for YAC72, and P<0.001 for YAC128) (Figure 6.4).

YAC128 HD animals display anhedonic behaviour

Anhedonia (inability to experience pleasure) is a major component of depression. Reduced sucrose intake and preference are considered a measure of anhedonia in mice (Willner et al., 1987). To determine whether YAC128 HD animals display anhedonic behaviour, the sucrose intake and preference of 3-4 months old YAC128 HD animals was measured over a 24 hr period. Consistent with anhedonic behaviour, YAC128 HD animals have a reduced sucrose intake compared to wildtype animals (P=0.0045) (Figure 6.5A). Furthermore, YAC128 animals exhibit reduced preference for sucrose compared to wildtype animals (Figure 6.5B), suggesting that the reduced sucrose consumption observed in these animals is not the result of generalized reduction in fluid intake. Indeed, there is no difference in the amount of water consumed between YAC128 HD and wildtype animals (Figure 6.5C).

YAC128 HD animals do not display anxiety-like behaviour

Anxiety or stress have been shown reproducibly to induce an acute increase in body temperature both in animals (Bouwknecht and Paylor, 2002; Zethof et al., 1994) and humans (Briese, 1995; Marazziti et al., 1992). This response, termed stress-induced hyperthermia, is well established as a measure of anxiety in animals (Zethof et al., 1994). This test was chosen as certain other tests of anxiety, such as the elevated plus/zero maze and the light/dark box, are influenced by visual cues (Cook et al., 2001; Wong and Brown, 2006) and FVB/N animals develop retinal degeneration and are impaired visually by weaning age (Chang et al., 2002). To examine whether YAC128 HD animals display anxiety-like behaviour, 3-4 months old YAC128 HD and wildtype animals were subjected to the SIH test. The body temperature of YAC128 HD animals,

measured rectally, was significantly increased during the SIH test (T1=36.67±0.11 vs. T2=37.04±0.09, P=0.017). Similarly, the body temperature of wildtype animals was significantly increased during the SIH test (T1=36.88±0.40 vs. T2=37.37±0.51, P=0.003). However, there was no difference in the SIH between YAC128 HD and wildtype animals (deltaT=0.37±0.10 for YAC128 and 0.49±0.13 for wildtype, P=0.432). This data suggests that depressive features but not signs of anxiety are seen in these mice.

Preventing cleavage of mutant huntingtin at residue 586 ameliorates the depressive behaviour observed in YAC128 HD animals

Proteolysis of mutant huntingtin has been shown to play an important role in the pathogenesis of HD. Prevention of cleavage at residue 586 of mutant huntingtin has been demonstrated to result in amelioration of motor dysfunction and striatal pathology in the transgenic YAC HD mice (Graham et al., 2006). To assess the effect of prevention of cleavage of mutant huntingtin at residue 586 on the depressive behaviour observed in YAC128 HD animals, WT and YAC128 animals along with YAC transgenic animals expressing a variant of mutant huntingtin that is resistant to cleavage at residue 586 (C6R) of 3-4 months of age were subjected to the FST and their immobility scored. YAC128 animals spent a significantly longer time in an immobile state compared to wildtype animals (P=0.005) (Figure 6.6A). In contrast, no significant difference in immobility between C6R and wildtype animals was observed (P=0.871). Furthermore, C6R animals spent significantly less time in an immobile state compared to YAC128 animals (P=0.035). Similarly, in the sucrose consumption test of anhedonia, while the intake of sucrose by YAC128 HD animals was significantly reduced compared to wildtype animals (P=0.010), no significant difference in sucrose intake between C6R and wildtype animals was observed (P=0.116) (Figure 6.6B). Furthermore, C6R animals consumed significantly more sucrose compared to YAC128 HD animals (P<0.001) (Figure 6.6B).

Anti-depressant treatment of YAC128 HD animals fails to ameliorate the depressive phenotype

To examine whether the depressive behaviour observed can be ameliorated by anti-depressant treatment, 4 months old YAC128 HD animals were treated with either fluoxetine or imipramine. Fluoxetine and imipramine treatment were used in the forced swim test and sucrose consumption test, respectively, since each is well established in the chosen concentration and the length of treatment as being efficacious in improving the depressive phenotype in the different tests (Duncan et al., 1996; Przegalinski et al., 1995). YAC128 animals were treated with saline or fluoxetine daily for 21 days and subjected to the forced swim test. Treatment of YAC128 animals with fluoxetine failed to decrease immobility time compared to saline-treated animals ($P=0.544$) (Figure 6.7A). Furthermore, there was no difference in the amount of time spent swimming or climbing by YAC128 HD animals following treatment with fluoxetine compared to saline (data not shown). Another group of YAC128 animals were treated with saline or imipramine daily for 21 days and their sucrose consumption was measured after 7, 14, and 21 days of treatment. There was no significant difference between saline- and imipramine-treated YAC128 HD animals in sucrose intake at any of the time points (Figure 6.7B).

6.4. Discussion

Affective disturbances, including depression, are highly prevalent amongst at-risk and symptomatic HD patients and contribute considerably to the morbidity in HD (recently reviewed in (van Duijn et al., 2007)). While the etiology of depression is thought to include pathophysiological changes caused by the mutation, this has not been directly examined in the absence of potential confounding psychosocial and environmental factors. Indeed, there is still controversy as to the nature and origin of depression in HD. In this respect, animal models provide the ideal tool in which to address this question under constant environmental conditions and in the absence of influence from psychosocial factors.

While there are obvious limitations in modeling psychiatric diseases in rodents (Cryan et al., 2005), considerable progress has been made in defining the molecular and physiological underpinnings of depressive syndromes in humans where close parallels in rodent models of depressive behaviour have been established. That such parallels exist provides some validity to the use of rodents in the study of depression.

In this study, we sought to determine whether the depressive behaviour observed in HD patients is reproduced in the transgenic YAC128 mouse model of HD. We demonstrate that the YAC128 HD animals display depressive behaviour in the FST at an early stage of the disease which does not worsen over time and is independent of CAG repeat length. The depressive phenotype in the YAC128 HD animals was further reproduced using a test of anhedonia, a key component of depression. We also demonstrate that YAC128 HD animals do not display anxiety-like behaviour. Furthermore, the depressive phenotypes are ameliorated in C6R animals expressing a variant of mutant huntingtin that is resistant to cleavage at residue 586. Finally, we demonstrate

that treatment with anti-depressants fails to ameliorate the depressive phenotype observed. Our findings provide strong support for a significant neurobiological contribution to depression in HD.

The severity of depressive behaviour is independent of disease stage and CAG repeat length

Unlike the HD-associated cognitive and motor deficits which worsen with the progression of the disease (Bamford et al., 1995; Ho et al., 2003; Lawrence et al., 1999), evidence from cross-sectional studies suggests that the severity of depression in HD is independent of disease progression (Berrios et al., 2001; Craufurd et al., 2001; Kingma et al., 2008). Consistent with findings in HD patients, we observed no significant difference in the severity of the depressive phenotype between 3, 8, and 12 months old YAC128 HD animals representing early and late phases of the illness in YAC128 mice (Slow et al., 2003; Van Raamsdonk et al., 2005b). This is in sharp contrast to the deficits in motor function observed in these animals which are progressive in nature and worsen with age (Slow et al., 2003; Van Raamsdonk et al., 2005b; Van Raamsdonk et al., 2005c).

Furthermore, while distinguishing apathy from depression in rodents is difficult, our data show no worsening in performance in the FST in YAC128 animals over time, which is consistent with these symptoms being reflective of depressive behaviour and not apathy. Indeed, apathy which is separable from depression (Levy et al., 1998) and is an important feature of HD (Folstein and Folstein, 1983), is found to correlate directly with disease stage and duration (Kingma et al., 2008; Craufurd et al., 2001).

The lack of correlation between depressive symptoms and disease severity in HD is markedly different than what is observed in other neurodegenerative disorders such as Parkinson's disease

where depressive symptoms are significantly related to illness severity (Brown et al., 1988; Cole et al., 1996). Furthermore, the clinical severity of depressive symptoms in adult-onset HD patients is independent of CAG repeat length (Andrew et al., 1993; Berrios et al., 2001; Close Kirkwood et al., 2002; Craufurd et al., 2001; MacMillan et al., 1993; Weigell-Weber et al., 1996; Zappacosta et al., 1996). These findings were paralleled in the YAC HD animals where the severity of the depressive phenotype was independent of CAG repeat length.

Increased immobility in the FST and reduced sucrose intake by YAC128 HD animals are independent of the motor dysfunction

It may be argued that since YAC128 HD animals develop progressive motor dysfunction starting at 3-4 months of age (Slow et al., 2003; Van Raamsdonk et al., 2005b; Van Raamsdonk et al., 2005c), that the increased immobility in the FST observed in the YAC128 HD mice may be the result of an impaired ability of YAC128 animals to swim. This possibility, however, is unlikely given that the ability of YAC128 HD animals to swim is not different from that of WT animals both in the early (3 months) and late (12 months) phases of disease, despite significant impairment in motor function (Figure 6.3). Furthermore, this is in agreement with our previous findings demonstrating that the cognitive deficits in the YAC128 HD animals as assessed by the swimming T-maze test of cognition is not due to any motor dysfunction-related impairment in swimming ability (Van Raamsdonk et al., 2005b).

Similarly, the reduced sucrose intake observed in YAC128 animals could reflect generalized reduction in drinking/fluid intake due to motor dysfunction. However, this is not the case as YAC128 HD animals show no reduction in water intake compared to wildtype animals but only a specific reduction in sucrose intake, as indicated by the lower sucrose preference score (Figure 6.4). Indeed, thirst has been shown to be increased in HD in advance stages of illness, and water

consumption is significantly higher in late-stage R6/2 mice compared to wildtype animals, despite profound motor deficits (Wood et al., 2008; Carter et al. 1993).

Anxiety-like behaviour is absent in YAC128 HD animals

The stress-induced hyperthermia test of anxiety is thought to reflect an unconditioned physiological response related to anticipatory anxiety (Van der Heyden et al., 1997), and has been validated using pharmacological tools including benzodiazepine treatment and novel anxiolytic drugs (Borsini et al., 1989; Van der Heyden et al., 1997). Our finding of lack of anxiety-like behaviour in YAC128 HD animals is consistent with observations in other animal models of HD (File et al., 1998; von Horsten et al., 2003). However, it may also be due to effects of background mouse strain. Indeed, differences in the extent of induced hyperthermia in the SIH test in a panel of inbred mouse strains have been demonstrated (Bouwknecht and Paylor, 2002). Interestingly, of 9 different animal strains tested, FVB/N mice showed the smallest induction of body temperature in this test, indicating least propensity to anxiety (Bouwknecht and Paylor, 2002). Thus, further assessment of anxiety-like behaviours in YAC128 animals on different animal strains could yield different results.

Prevention of cleavage of mutant huntingtin at residue 586 ameliorates the depressive behaviour in YAC128 HD animals

We have shown previously that eliminating cleavage of mutant huntingtin at residue 586 in C6R animals is sufficient to preserve striatal volume and rescue cognitive and motor function in the YAC128 mouse model of HD (Graham et al., 2006). We now show that the depressive phenotypes observed in the YAC128 HD animals are also ameliorated by preventing cleavage of mutant huntingtin at residue 586 in C6R animals. The cleavage of mutant huntingtin yielding a 586 aa fragment, which is detected in the nucleus and is an early event (Warby et al., 2008), is associated with neuropathology and motor deficits in vivo. This fragment is absent in C6R

animals, and therefore may be a key and rate limiting step underlying not only motor and neuronal deficits, but also the psychiatric disturbance observed in HD. Strategies aimed at modulating mutant huntingtin proteolysis may therefore also be of therapeutic value in the management of the psychiatric disturbance in HD.

Variability of expression of depression in HD

Depression has been reported to occur in about 40% to 50% of HD patients (Duff et al., 2007; Folstein and Folstein, 1983; Heathfield, 1967; Kirkwood et al., 2001; Pflanz et al., 1991). Development of depression can result from a broad range of genetic and environmental factors that together confer vulnerability. In the presence of psychosocial stressors, these factors precipitate depressive syndromes (Brown et al., 1986; Billings et al., 1983). This complex interaction of genetic and environmental susceptibility factors is likely to account for the variability of expression of depression in HD. Indeed, one may view mutant *HTT* as one important genetic risk factor predisposing individuals to increased risk of depressive behaviour, which in the presence of other precipitating genetic factors and environmental stressors leads to depression.

Early disruption of neural circuits likely underlies the psychiatric disturbances in HD

Increasing evidence points to alterations in neuronal plasticity and the consequent disruption in neural circuitry and gene expression as key mechanisms underlying major depressive disorders (Manji et al., 2001; McClung and Nestler, 2008; Pittenger and Duman, 2008). Several findings in HD patients and animal models of HD seem to suggest that a similar mechanism may account for the depression phenotype observed in HD. Indeed, multiple abnormalities in the dopaminergic, cholinergic, and glutamatergic signaling systems, which are integral components of the synaptic plasticity machinery, have been observed in HD (Di Filippo et al., 2007; Li et al., 2003). For

example, YAC46 and YAC72 HD mice exhibit early electrophysiological abnormalities indicative of altered synaptic function, including NMDA receptor hyperactivity, that can be detected prior to neurodegeneration (Hodgson et al., 1999). In addition, several abnormalities exist in NMDA and AMPA receptor-mediated corticostriatal synaptic signaling in YAC72 and YAC128 HD animals, which predate the detection of motor or cognitive deficits (Milnerwood and Raymond, 2007). These changes are consistent with the alterations in the NMDA receptor signaling machinery observed in HD patients (Young et al., 1988). Furthermore, the expression of dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), a major component in dopaminergic signaling and a potential mediator of the molecular effects of the anti-depressant fluoxetine (Svenningsson et al., 2002), is significantly decreased in YAC128 HD animals (Van Raamsdonk et al., 2005). This is in agreement with alterations in the dopamine signaling pathways in HD patients (Pavese et al., 2003; van Oostrom et al., 2005). Similar observations of altered synaptic plasticity have been observed in other animal models of HD (Di Filippo et al., 2007).

Perturbations in additional pathways known to influence synaptic plasticity are also well documented. These include alterations in the BDNF system (Castren et al., 2007; Martinowich et al., 2007) and neuroinflammatory activation (Dunn et al., 2005; Raison et al., 2006; Stellwagen and Malenka, 2006; Todd et al., 2006) which have been documents in HD patients and YAC128 animals (Ferrer et al., 2000; Strand et al., 2007; Zuccato and Cattaneo, 2007; Zuccato et al., 2001; Dalrymple et al., 2007; Tai et al., 2007; Bjorkqvist et al., 2008). Finally, overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, which is thought to play a role in depression (Muller and Holsboer, 2006), has been observed in HD patients and is likely to contribute to the depressive phenotype (Bjorkqvist et al., 2006; Petersen and Bjorkqvist, 2006). These alterations

may collectively contribute to disrupted synaptic plasticity and the psychiatric disturbances in HD.

Further evidence in support of disrupted neural circuitry in HD is provided by studies demonstrating significantly reduced glucose metabolism in the basal ganglia and cortical brain regions of HD patients compared to controls (Martin et al., 1992). Consistent with the early occurrence of depressive symptoms in HD, the hypometabolism is observed early and precedes neuronal loss (Hayden et al., 1986; Kuhl et al., 1982). In particular, selective hypometabolism in the paralimbic frontal lobe region was found in depressed patients with HD compared to non-depressed patients and normal controls, implicating dysfunction of neural circuits involving the paralimbic regions of the frontal lobes in the depressive symptoms in HD (Mayberg et al., 1992).

Thus it is likely that these multi-system alterations contribute to the disruption of neural circuitry and, in conjunction with predisposing environmental and genetic factors, lead to psychiatric disturbances in HD. The extent to which the neural circuitry is affected may account for the heterogeneity observed in the timing and nature of the psychiatric symptoms. Similar variations in perturbations of neural circuitry may also occur in depressive disorders in general. The YAC128 HD animals did not show improvement in depressive symptoms following anti-depressant treatment, which is similar to what has been observed with the responsiveness of the depressive phenotype in certain other models of neurological disorder such as epilepsy (Mazarati et al., 2008). Indeed, only 50% of individuals with depression show full remission with optimized treatment with current available antidepressant therapy (Berton and Nestler, 2006). No definitive trial of antidepressant treatment in HD has been conducted. Furthermore, considerable variability in responsiveness to anti-depressant treatment in HD patients has been reported (Leroi and Michalon, 1998).

Chronic treatment with antidepressants of the selective serotonin reuptake inhibitor (SSRI) class has been shown to lead to increased brain BDNF levels (Nibuya et al., 1995). In light of the deficits in brain BDNF levels in HD (Zuccato and Cattaneo, 2007), treatment with such SSRIs in HD may be neuroprotective. Indeed, treatment with the SSRI sertraline has recently been shown to increase survival, improve motor deficits and attenuate the progression of brain atrophy in a mouse model of HD (Duan et al., 2008).

While in the final stages of revision of this manuscript, a study was published examining depressive behaviour in the R6/1 mouse model of HD (Pang et al., 2008). Female, but not male, transgenic R6/1 HD animals were found to exhibit depressive behaviour as assessed by the FST. Treatment with the anti-depressant sertraline ameliorated the depressive phenotype in females. This is in contrast to our findings of depressive behaviour in male and female YAC128 animals and no improvement following antidepressant treatment. The two models express different constructs of huntingtin with R6/1 animals expressing exon 1 and YAC128 animals expressing full-length human huntingtin, which may partly account for these differences. Additional differences in the background strain may underlie these varying results.

Here, we demonstrate depressive behaviour in the transgenic YAC128 mouse model of HD that adds to the motor and cognitive deficits and selective neuropathology present in these animals (Figure 6.8). The depressive behaviour occurs early, does not worsen with time, and is ameliorated by prevention of cleavage of mutant huntingtin at residue 586. The depressive behaviour recapitulates that observed in human HD (Table 2-1) and can be used as an outcome measure in therapeutic trials to assess the effect of potential treatments on depression.

Table 6-1 Comparison of the Characteristics of the Depressive Behaviour in HD Patients and YAC128 HD Animals

	Human HD Patients	**YAC128 HD Mice**
Time of presentation of depressive symptoms	Early (Duff et al., 2007; Folstein and Folstein, 1983; Heathfield, 1967)	Early (Figure 6.1)
Severity in relation to disease (motor) stage/age	Independent/Does not worsen with age (Craufurd et al., 2001; Kingma et al., 2008)	Independent/Does not worsen with age (Figure 6.2)
Relationship to CAG repeat length	Independent of CAG repeat length (Andrew et al., 1993; Berrios et al., 2001; Close Kirkwood et al., 2002; Craufurd et al., 2001; MacMillan et al., 1993; Weigell-Weber et al., 1996)	Independent of CAG repeat length (Figure 6.4)

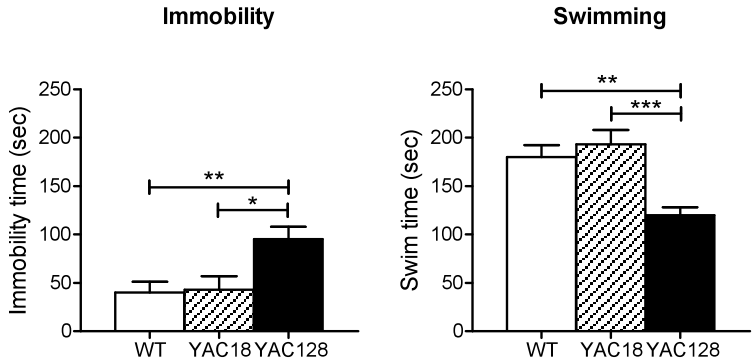


Figure 6.1 YAC128 HD animals display depressive behaviour

YAC128, YAC18, and wildtype animals were subjected to the forced swim test at 3 months of age. YAC128 animals spent a significantly longer time in an immobile state compared to YAC18 (P=0.027) and wildtype animals (P=0.008). Consistent with the increased immobility displayed, YAC128 animals spent significantly less time swimming compared to YAC18 (P<0.001) and wildtype animals (P=0.002). No significant differences in immobility or swimming behaviours between YAC18 and wildtype animals were observed (P=0.986, and P=0.733, respectively). Data are represented as means±SEM; n=12 (WT), 8 (YAC18), and 12 (YAC128); females. ** P < 0.01, *** P < 0.001.

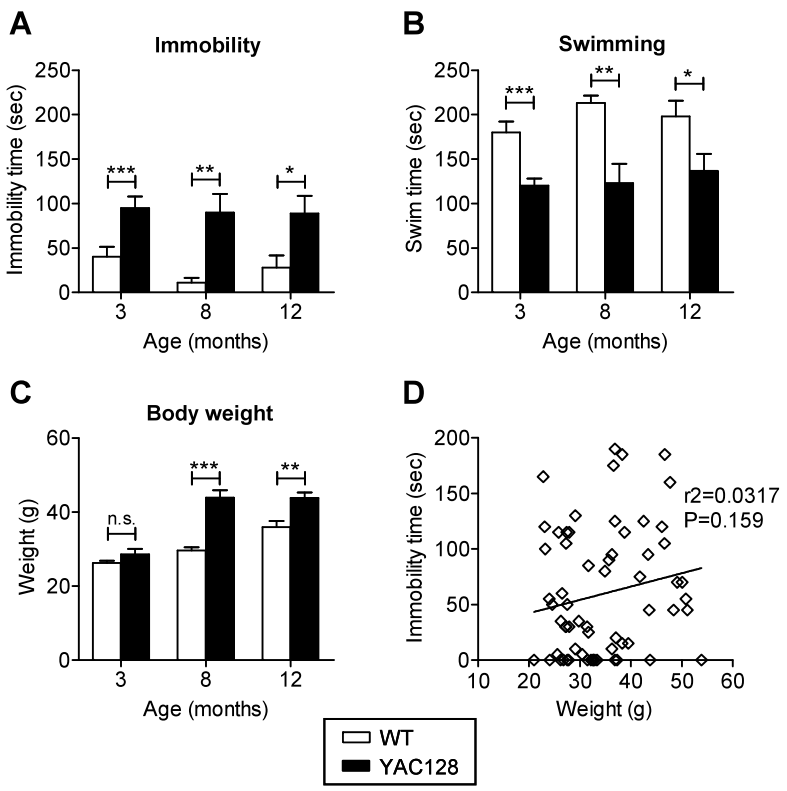


Figure 6.2 The severity of the depressive behaviour does not increase over time and is independent of animal body weight

YAC128 animals and WT littermates were subjected to the forced swim test at 8 and 12 months of age. (A) As seen at 3 months of age, YAC128 animals spent a significantly longer time in an immobile state compared to WT littermates (P=0.002 and 0.0187 for 8 and 12 months old mice, respectively) and (B) significantly less time swimming (P=0.001 and 0.0295 for 8 and 12 months old mice, respectively). No significant differences were observed between 3, 8, and 12 months-old YAC128 animals in immobility (P=0.965) or swimming times (P=0.727). Similarly, no significant differences were observed between 3, 8, and 12 months-old WT animals in immobility (P=0.218) or swimming times (P=0.267) (C) The body weight of 3 months old YAC128 animals does not differ from that of wildtype littermates (P=0.135), while increased body weight compared to WT littermates is seen in 8 months old (P<0.001) and 12 months old YAC128 animals (P=0.0026). (D) To examine whether increased body weight may contribute to immobility in the forced swim test, immobility time of 3, 8, and 12-months old animals was plotted against body weight. Regression analysis revealed no correlation between body weight and immobility time (r2=0.0317, P=0.159). Data are represented as means±SEM; for (A-C) n=12 (3 months), 9 (8 months), 11 (12 months); for (D) Individual animals are represented by diamond shapes. n=64. ** P < 0.01, *** P < 0.001, n.s.=no significant difference.

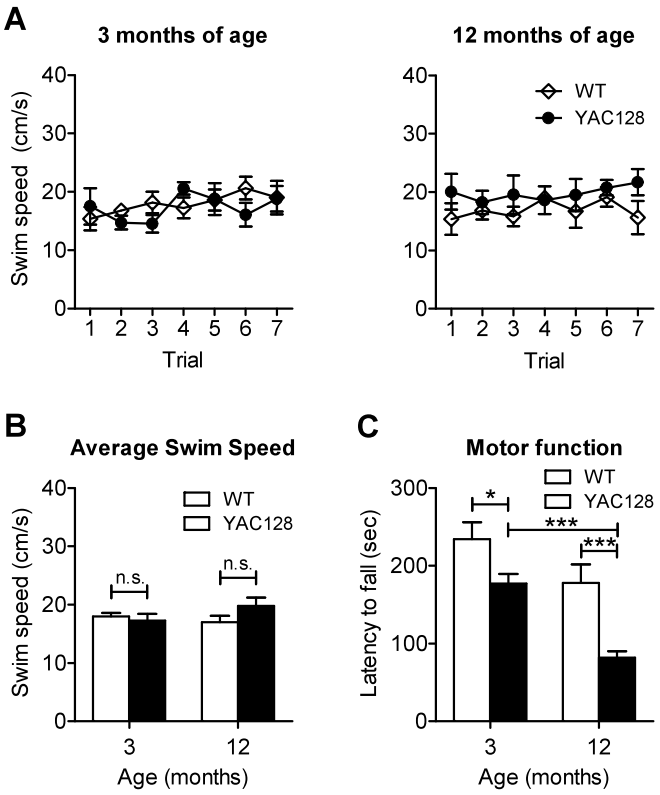


Figure 6.3 The ability to swim is not impaired in the YAC128 HD animals despite motor dysfunction

To assess whether the ability to swim in YAC128 HD animals is impaired, animals were tested in a simple swimming test. In this test, mice were placed at one end of a linear swimming chamber and trained to reach a platform at the other end of the chamber to escape from the water. Mice were tested with seven successive trials and the swimming speed for each mouse was recorded for each trial. (A) There was no significant difference in the swimming speed of YAC128 animals compared to WT animals at either 3 or 12 months of age in any of the trials. (B) The average swimming speed over the 7 trials was not significantly different between YAC128 and WT animals at either 3 or 12 months of age, despite significantly lower performance by YAC128 animals in the rotarod test of motor function compared to WT animals (C). Data are represented as means±SEM; for (A-B) n=5 WT and 8 YAC128 (3 months time-point) and n=8 WT and 8 YAC128 (12 months time-point); for (C) n=12 WT and 17 YAC128; n.s.=no significant difference. * $P < 0.05$, *** $P < 0.001$.

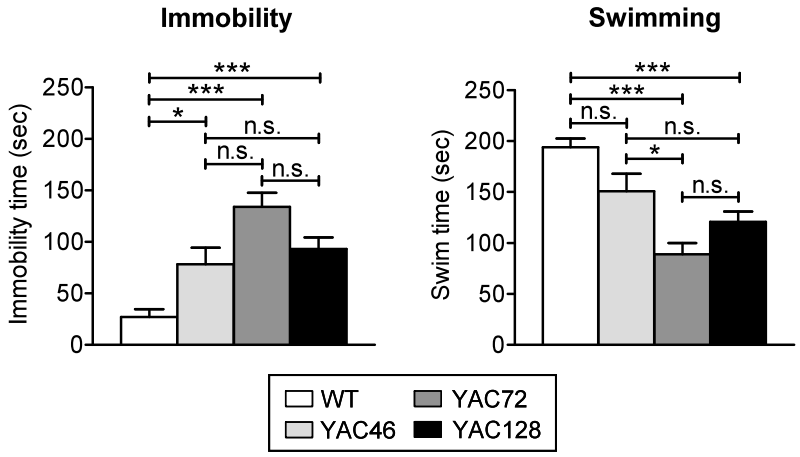


Figure 6.4 The severity of the depressive behaviour in YAC transgenic HD animals is independent of CAG repeat length

To assess whether the severity of the depressive behaviour correlated with CAG repeat length, wildtype, YAC46, YAC72, and YAC128 animals were subjected to the forced swim test. Similar to YAC128 animals, YAC46 and YAC72 animals were immobile for significantly longer times than wildtype animals (vs. wildtype: P=0.026 for YAC46, <0.001 for YAC72, and <0.001 for YAC128). No difference in the extent of immobility between YAC46, YAC72, and YAC128 animals was observed (YAC46 vs. YAC72 and YAC128: P=0.054 and 0.828, respectively; YAC72 vs. YAC128: P=0.125). YAC46, YAC72, and YAC128 animals also spent less time swimming compared to wildtype animals, although the difference did not reach statistical significance in the case of the YAC46 animals (vs. wildtype: P=0.067 for YAC46, <0.001 for YAC72, and <0.001 for YAC128). Data are represented as means±SEM; n=21 (WT), 9 (YAC46), 8 (YAC72), and 21 (YAC128). One-way ANOVA with Tukey post-hoc test; * P < 0.05, *** P < 0.001, n.s.=no significant difference.

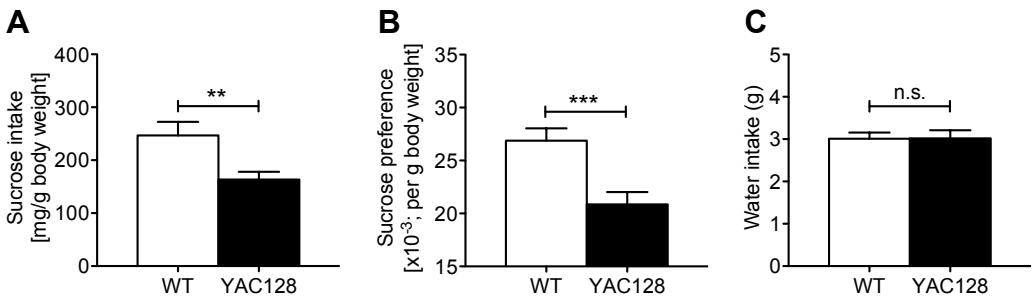


Figure 6.5 YAC128 HD animals display anhedonic behaviour

An inability to experience pleasure is considered a central aspect of depressive behaviour. To examine whether YAC128 animals display anhedonic behaviour, the sucrose intake of 3-4 months old YAC128 animals over a 24-hr period was measured. (A) YAC128 animals consumed significantly less sucrose solution compared to WT animals (P=0.0045). (B) YAC128 animals exhibited significantly reduced preference for sucrose solution compared to WT animals (P=0.0004). (C) No difference in water intake was observed between YAC128 HD and WT animals (P=0.492). Data are represented as means±SEM; n=26 for WT, 23 for YAC128. ** P < 0.01; *** P < 0.001, n.s.=no significant difference.

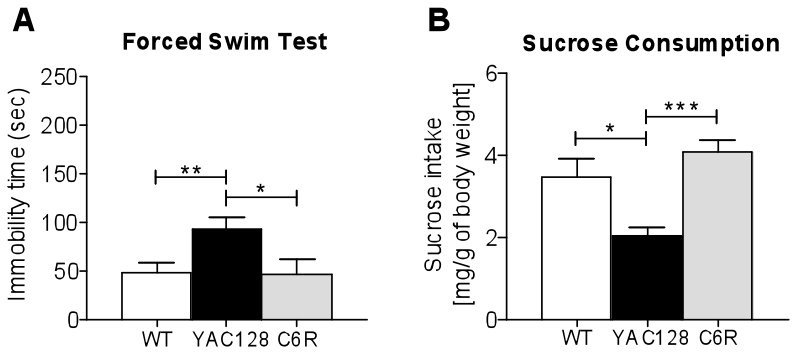


Figure 6.6 Preventing cleavage of mutant huntingtin at residue 586 ameliorates the depressive behaviour in YAC128 HD animals

To assess the effect of prevention of cleavage at residue 586 of mutant huntingtin on the depressive behaviour observed in YAC128 HD animals, WT and YAC128 animals along with YAC transgenic animals expressing a variant of mutant huntingtin that is resistant to cleavage at residue 586 (C6R) were subjected to the forced swim test and their sucrose consumption was measured at 3-4 months of age. (A) In the forced swim test, YAC128 animals spent a significantly longer time in an immobile state compared to wildtype animals (P=0.005). In contrast, no significant difference in immobility between C6R and wildtype animals was observed (P=0.871). Furthermore, C6R animals spent significantly less time in an immobile state compared to YAC128 animals (P=0.035). (B) YAC128 animals consumed significantly less sucrose per gram of body weight compared to wildtype animals (P=0.010). In contrast, no significant difference in sucrose intake per gram of body weight between C6R and wildtype animals was observed (P=0.116). Furthermore, C6R animals consumed significantly more sucrose per gram of body weight compared to YAC128 animals (P<0.001). Data are represented as means±SEM; * P < 0.05; ** P < 0.01; *** P < 0.001.

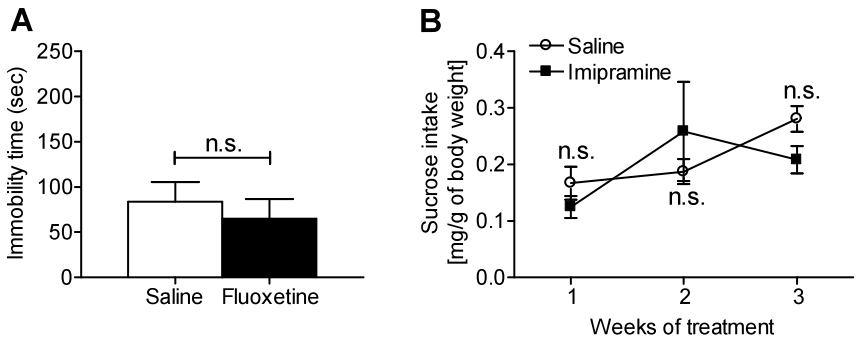


Figure 6.7 Anti-depressant treatment fails to ameliorate the depressive phenotype in the YAC128 HD animals

To examine whether the depressive behaviour observed can be modulated, YAC128 animals were treated with the antidepressants. Fluoxetine and imipramine treatment were used in the forced swim test and sucrose consumption test, respectively, since each is well established as being efficacious in improving the depressive phenotype in the respective test. (A) YAC128 animals were treated with saline or 20mg/kg of fluoxetine intraperitoneally, daily for 21 days, and subjected to the forced swim test. Treatment of YAC128 animals with fluoxetine failed to decrease immobility time compared to saline-treated animals (P=0.544). (B) YAC128 animals were treated with saline or 10mg/kg of imipramine b.i.d. intraperitoneally, daily for 21 days and their sucrose consumption was measured after 7, 14, and 21 days of treatment. There was no significant difference between saline- and imipramine-treated YAC128 animals in sucrose intake at any of the time points. Data are represented as means±SEM; n=9 for (A), 5 for (B). n.s.=no significant difference.

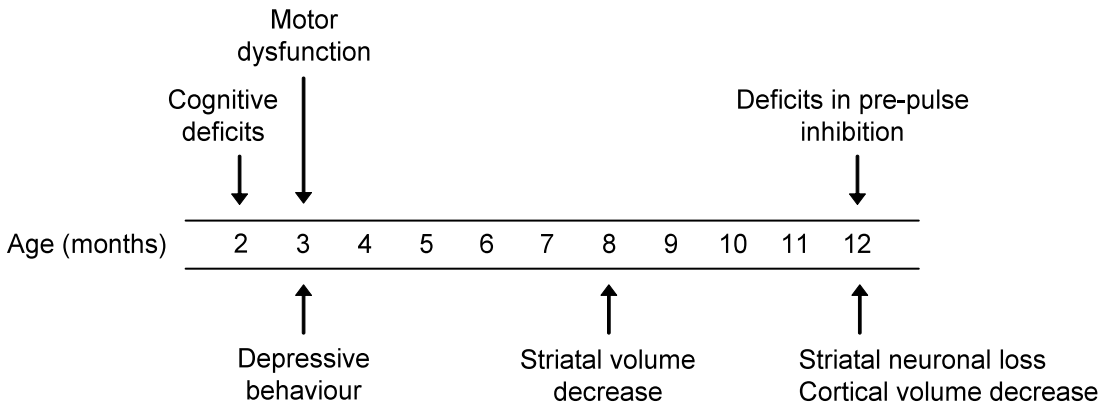


Figure 6.8 Time-course of behavioural and neuropathological correlates of HD in the YAC128 animals

YAC128 animals recapitulates several features of human HD including progressive cognitive deficits starting at 2 months of age, motor dysfunction and depressive behaviour by 3 months of age, followed by selective neuropathology with striatal atrophy clearly evident by 8 months of age and cortical volume loss by 12 months of age. Deficits in pre-pulse inhibition are seen at 12 months of age.

6.5. References

Andrew, S.E., Goldberg, Y.P., Kremer, B., Telenius, H., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M.A., Graham, R.K., Hayden, M.R., 1993. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet. 4, 398-403.

Bamford, K.A., Caine, E.D., Kido, D.K., Cox, C., Shoulson, I., 1995. A prospective evaluation of cognitive decline in early Huntington's disease: functional and radiographic correlates. Neurology. 45, 1867-73.

Berrios, G.E., Wagle, A.C., Marková, I.S., Wagle, S.A., Ho, L.W., Rubinsztein, D.C., Whittaker, J., Ffrench-Constant, C., Kershaw, A., Rosser, A., Bak, T., Hodges, J.R., 2001. Psychiatric symptoms and CAG repeats in neurologically asymptomatic Huntington's disease gene carriers. Psychiatry Res. 102, 217-25.

Berton, O., Nestler, E.J., 2006. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 7, 137-51.

Billings, A.G., Cronkite, R.C., Moos, R.H., 1983. Social-environmental factors in unipolar depression: comparisons of depressed patients and nondepressed controls. J Abnorm Psychol. 92, 119-33.

Björkqvist, M., Petersén, A., Bacos, K., Isaacs, J., Norlén, P., Gil, J., Popovic, N., Sundler, F., Bates, G.P., Tabrizi, S.J., Brundin, P., Mulder, H., 2006. Progressive alterations in the hypothalamic-pituitary-adrenal axis in the R6/2 transgenic mouse model of Huntington's disease. Hum Mol Genet. 15, 1713-21.

Björkqvist, M., Wild, E.J., Thiele, J., Silvestroni, A., Andre, R., Lahiri, N., Raibon, E., Lee, R.V., Benn, C.L., Soulet, D., Magnusson, A., Woodman, B., Landles, C., Pouladi, M.A., Hayden, M.R., Khalili-Shirazi, A., Lowdell, M.W., Brundin, P., Bates, G.P., Leavitt, B.R., Möller, T., Tabrizi, S.J., 2008. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med. 205, 1869-77.

Borsini, F., Lecci, A., Volterra, G., Meli, A., 1989. A model to measure anticipatory anxiety in mice? Psychopharmacology. 98, 207-11.

Bouwknecht, J.A., Paylor, R., 2002. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behav Brain Res. 136, 489-501.

Briese, E., 1995. Emotional hyperthermia and performance in humans. Physiol Behav. 58, 615-8.

Brown, G.W., Bifulco, A., Harris, T., Bridge, L., 1986. Life stress, chronic subclinical symptoms and vulnerability to clinical depression. J Affect Disord. 11, 1-19.

Brown, R.G., MacCarthy, B., Gotham, A.M., Der, G.J., Marsden, C.D., 1988. Depression and disability in Parkinson's disease: a follow-up of 132 cases. Psychol Med. 18, 49-55.

Carter, R.J., Lione, L.A., Humby, T., Mangiarini, L., Mahal, A., Bates, G.P., Dunnett, S.B., Morton, A.J., 1999. Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci. 19, 3248-57.

Castrén, E., Võikar, V., Rantamäki, T., 2007. Role of neurotrophic factors in depression. Current opinion in pharmacology. 7, 18-21.

Chang, B., Hawes, N.L., Hurd, R.E., Davisson, M.T., Nusinowitz, S., Heckenlively, J.R., 2002. Retinal degeneration mutants in the mouse. Vision Res. 42, 517-25.

Close Kirkwood, S., Siemers, E., Viken, R.J., Hodes, M.E., Conneally, P.M., Christian, J.C., Foroud, T., 2002. Evaluation of psychological symptoms among presymptomatic HD gene carriers as measured by selected MMPI scales. Journal of psychiatric research. 36, 377-82.

Cole, S.A., Woodard, J.L., Juncos, J.L., Kogos, J.L., Youngstrom, E.A., Watts, R.L., 1996. Depression and disability in Parkinson's disease. The Journal of neuropsychiatry and clinical neurosciences. 8, 20-5.

Cook, M.N., Williams, R.W., Flaherty, L., 2001. Anxiety-related behaviors in the elevated zero-maze are affected by genetic factors and retinal degeneration. Behav Neurosci. 115, 468-76.

Craufurd, D., Thompson, J.C., Snowden, J.S., 2001. Behavioral changes in Huntington Disease. Neuropsychiatry, neuropsychology, and behavioral neurology. 14, 219-26.

Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci. 23, 238-45.

Cryan, J.F., Holmes, A., 2005. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov. 4, 775-90.

Dalrymple, A., Wild, E.J., Joubert, R., Sathasivam, K., Björkqvist, M., Petersén, Å., Jackson, G.S., Isaacs, J.D., Kristiansen, M., Bates, G.P., Leavitt, B.R., Keir, G., Ward, M., Tabrizi, S.J., 2007. Proteomic Profiling of Plasma in Huntington's Disease Reveals Neuroinflammatory Activation and Biomarker Candidates. J. Proteome Res. 6, 2833-2840.

Di Filippo, M., Tozzi, A., Picconi, B., Ghiglieri, V., Calabresi, P., 2007. Plastic abnormalities in experimental Huntington's disease. Current opinion in pharmacology. 7, 106-11.

Duan, W., Peng, Q., Masuda, N., Ford, E., Tryggestad, E., Ladenheim, B., Zhao, M., Cadet, J.L., Wong, J., Ross, C.A., 2008. Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease. Neurobiology of Disease. 30, 312-22.

Duff, K., Paulsen, J.S., Beglinger, L.J., Langbehn, D.R., Stout, J.C., Group, P.-H.I.o.t.H.S., 2007. Psychiatric symptoms in Huntington's disease before diagnosis: the predict-HD study. Biol Psychiatry. 62, 1341-6.

Duncan, G.E., Knapp, D.J., Johnson, K.B., Breese, G.R., 1996. Functional classification of antidepressants based on antagonism of swim stress-induced fos-like immunoreactivity. J Pharmacol Exp Ther. 277, 1076-89.

Dunn, A.J., Swiergiel, A.H., de Beaurepaire, R., 2005. Cytokines as mediators of depression: what can we learn from animal studies? Neuroscience and biobehavioral reviews. 29, 891-909.

Farrer, L.A., 1986. Suicide and attempted suicide in Huntington disease: implications for preclinical testing of persons at risk. Am J Med Genet. 24, 305-11.

File, S.E., Mahal, A., Mangiarini, L., Bates, G.P., 1998. Striking changes in anxiety in Huntington's disease transgenic mice. Brain Res. 805, 234-40.

Folstein, S.E., Folstein, M.F., 1983. Psychiatric features of Huntington's disease: recent approaches and findings. Psychiatr Dev. 1, 193-205.

Graham, R.K., Deng, Y., Slow, E.J., Haigh, B., Bissada, N., Lu, G., Pearson, J., Shehadeh, J., Bertram, L., Murphy, Z., Warby, S.C., Doty, C.N., Roy, S., Wellington, C.L., Leavitt, B.R., Raymond, L.A., Nicholson, D.W., Hayden, M.R., 2006. Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell. 125, 1179-91.

Hayden, M.R., 1981. Huntington's chorea, Vol., Springer-Verlag, Berlin; New York.

Hayden, M.R., Martin, W.R., Stoessl, A.J., Clark, C., Hollenberg, S., Adam, M.J., Ammann, W., Harrop, R., Rogers, J., Ruth, T., 1986. Positron emission tomography in the early diagnosis of Huntington's disease. Neurology. 36, 888-94.

Heathfield, K.W., 1967. Huntington's chorea. Investigation into the prevalence of this disease in the area covered by the North East Metropolitan Regional Hospital Board. Brain. 90, 203-32.

Ho, A.K., Sahakian, B.J., Brown, R.G., Barker, R.A., Hodges, J.R., Ané, M.-N., Snowden, J., Thompson, J., Esmonde, T., Gentry, R., Moore, J.W., Bodner, T., Consortium, N.-H., 2003. Profile of cognitive progression in early Huntington's disease. Neurology. 61, 1702-6.

Hodgson, J.G., Smith, D.J., McCutcheon, K., Koide, H.B., Nishiyama, K., Dinulos, M.B., Stevens, M.E., Bissada, N., Nasir, J., Kanazawa, I., Disteche, C.M., Rubin, E.M., Hayden, M.R., 1996. Human huntingtin derived from YAC transgenes compensates for loss of murine huntingtin by rescue of the embryonic lethal phenotype. Hum Mol Genet. 5, 1875-85.

Hodgson, J.G., Agopyan, N., Gutekunst, C.A., Leavitt, B.R., LePiane, F., Singaraja, R., Smith, D.J., Bissada, N., McCutcheon, K., Nasir, J., Jamot, L., Li, X.J., Stevens, M.E., Rosemond, E., Roder, J.C., Phillips, A.G., Rubin, E.M., Hersch, S.M., Hayden, M.R., 1999. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron. 23, 181-92.

Julien, C.L., Thompson, J.C., Wild, S., Yardumian, P., Snowden, J.S., Turner, G., Craufurd, D., 2007. Psychiatric disorders in preclinical Huntington's disease. J Neurol Neurosurg Psychiatr. 78, 939-43.

Kingma, E.M., van Duijn, E., Timman, R., van der Mast, R.C., Roos, R.A.C., 2008. Behavioural problems in Huntington's disease using the Problem Behaviours Assessment. General hospital psychiatry. 30, 155-61.

Kirkwood, S.C., Su, J.L., Conneally, P., Foroud, T., 2001. Progression of symptoms in the early and middle stages of Huntington disease. Arch Neurol. 58, 273-8.

Kuhl, D.E., Phelps, M.E., Markham, C.H., Metter, E.J., Riege, W.H., Winter, J., 1982. Cerebral metabolism and atrophy in Huntington's disease determined by 18FDG and computed tomographic scan. Ann Neurol. 12, 425-34.

Lawrence, A.D., Sahakian, B.J., Rogers, R.D., Hodge, J.R., Robbins, T.W., 1999. Discrimination, reversal, and shift learning in Huntington's disease: mechanisms of impaired response selection. Neuropsychologia. 37, 1359-74.

Lerch, J.P., Carroll, J.B., Spring, S., Bertram, L.N., Schwab, C., Hayden, M.R., Henkelman, R.M., 2008. Automated deformation analysis in the YAC128 Huntington disease mouse model. Neuroimage. 39, 32-9.

Leroi, I., Michalon, M., 1998. Treatment of the psychiatric manifestations of Huntington's disease: a review of the literature. Canadian journal of psychiatry Revue canadienne de psychiatrie. 43, 933-40.

Levy, M.L., Cummings, J.L., Fairbanks, L.A., Masterman, D., Miller, B.L., Craig, A.H., Paulsen, J.S., Litvan, I., 1998. Apathy is not depression. The Journal of neuropsychiatry and clinical neurosciences. 10, 314-9.

Li, J.-Y., Plomann, M., Brundin, P., 2003. Huntington's disease: a synaptopathy? Trends in molecular medicine. 9, 414-20.

Lieberman, A., 2006. Depression in Parkinson's disease -- a review. Acta Neurol Scand. 113, 1-8.

Litvan, I., Cummings, J.L., Mega, M., 1998. Neuropsychiatric features of corticobasal degeneration. J Neurol Neurosurg Psychiatr. 65, 717-21.

MacMillan, J.C., Snell, R.G., Tyler, A., Houlihan, G.D., Fenton, I., Cheadle, J.P., Lazarou, L.P., Shaw, D.J., Harper, P.S., 1993. Molecular analysis and clinical correlations of the Huntington's disease mutation. Lancet. 342, 954-8.

Manji, H.K., Drevets, W.C., Charney, D.S., 2001. The cellular neurobiology of depression. Nat Med. 7, 541-7.

Marazziti, D., Di Muro, A., Castrogiovanni, P., 1992. Psychological stress and body temperature changes in humans. Physiol Behav. 52, 393-5.

Marshall, J., White, K., Weaver, M., Flury Wetherill, L., Hui, S., Stout, J.C., Johnson, S.A., Beristain, X., Gray, J., Wojcieszek, J., Foroud, T., 2007. Specific psychiatric manifestations among preclinical Huntington disease mutation carriers. Arch Neurol. 64, 116-21.

Martin, W.R., Clark, C., Ammann, W., Stoessl, A.J., Shtybel, W., Hayden, M.R., 1992. Cortical glucose metabolism in Huntington's disease. Neurology. 42, 223-9.

Martinowich, K., Manji, H., Lu, B., 2007. New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10, 1089-93.

Mayberg, H.S., Starkstein, S.E., Peyser, C.E., Brandt, J., Dannals, R.F., Folstein, S.E., 1992. Paralimbic frontal lobe hypometabolism in depression associated with Huntington's disease. Neurology. 42, 1791-7.

Mazarati, A., Siddarth, P., Baldwin, R.A., Shin, D., Caplan, R., Sankar, R., 2008. Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. Brain. 131, 2071-83.

Mcclung, C.A., Nestler, E.J., 2008. Neuroplasticity mediated by altered gene expression. Neuropsychopharmacology. 33, 3-17.

Milnerwood, A.J., Raymond, L.A., 2007. Corticostriatal synaptic function in mouse models of Huntington's disease: early effects of huntingtin repeat length and protein load. The Journal of Physiology. 585, 817-831.

Müller, M.B., Holsboer, F., 2006. Mice with mutations in the HPA-system as models for symptoms of depression. Biol Psychiatry. 59, 1104-15.

Pang, T.Y.C., Du, X., Zajac, M.S., Howard, M.L., Hannan, A.J., 2009. Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. Hum Mol Genet. 18, 753-66.

Pavese, N., Andrews, T.C., Brooks, D.J., Ho, A.K., Rosser, A.E., Barker, R.A., Robbins, T.W., Sahakian, B.J., Dunnett, S.B., Piccini, P., 2003. Progressive striatal and cortical dopamine receptor dysfunction in Huntington's disease: a PET study. Brain. 126, 1127-35.

Petersén, Å., Björkqvist, M., 2006. Hypothalamic-endocrine aspects in Huntington's disease. Eur J Neurosci. 24, 961-967.

Pflanz, S., Besson, J.A., Ebmeier, K.P., Simpson, S., 1991. The clinical manifestation of mental disorder in Huntington's disease: a retrospective case record study of disease progression. Acta psychiatrica Scandinavica. 83, 53-60.

Pittenger, C., Duman, R.S., 2008. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 33, 88-109.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977a. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 229, 327-36.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977b. Depression: a new animal model sensitive to antidepressant treatments. Nature. 266, 730-2.

Przegaliński, E., Moryl, E., Papp, M., 1995. The effect of 5-HT1A receptor ligands in a chronic mild stress model of depression. Neuropharmacology. 34, 1305-10.

Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24-31.

Slow, E.J., van Raamsdonk, J., Rogers, D., Coleman, S.H., Graham, R.K., Deng, Y., Oh, R., Bissada, N., Hossain, S.M., Yang, Y.-Z., Li, X.-J., Simpson, E.M., Gutekunst, C.-A., Leavitt, B.R., Hayden, M.R., 2003. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet. 12, 1555-67.

Stellwagen, D., Malenka, R.C., 2006. Synaptic scaling mediated by glial TNF-alpha. Nature. 440, 1054-9.

Strand, A.D., Baquet, Z.C., Aragaki, A.K., Holmans, P., Yang, L., Cleren, C., Beal, M.F., Jones, L., Kooperberg, C., Olson, J.M., Jones, K.R., 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, 11758-68.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F.A., Gass, P., 2004. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology. 29, 2007-17.

Svenningsson, P., Tzavara, E.T., Witkin, J.M., Fienberg, A.A., Nomikos, G.G., Greengard, P., 2002. Involvement of striatal and extrastriatal DARPP-32 in biochemical and behavioral effects of fluoxetine (Prozac). Proc Natl Acad Sci U S A. 99, 3182-7.

Tai, Y.F., Pavese, N., Gerhard, A., Tabrizi, S.J., Barker, R.A., Brooks, D.J., Piccini, P., 2007. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain. 130, 1759-66.

Todd, K.J., Serrano, A., Lacaille, J.-C., Robitaille, R., 2006. Glial cells in synaptic plasticity. J Physiol Paris. 99, 75-83.

Van der Heyden, J.A., Zethof, T.J., Olivier, B., 1997. Stress-induced hyperthermia in singly housed mice. Physiol Behav. 62, 463-70.

van Duijn, E., Kingma, E.M., van der Mast, R.C., 2007. Psychopathology in verified Huntington's disease gene carriers. The Journal of neuropsychiatry and clinical neurosciences. 19, 441-8.

van Oostrom, J.C.H., Maguire, R.P., Verschuuren-Bemelmans, C.C., Veenma-van der Duin, L., Pruim, J., Roos, R.A.C., Leenders, K.L., 2005. Striatal dopamine D2 receptors, metabolism, and volume in preclinical Huntington disease. Neurology. 65, 941-3.

Van Raamsdonk, J.M., Pearson, J., Bailey, C.D.C., Rogers, D.A., Johnson, G.V.W., Hayden, M.R., Leavitt, B.R., 2005a. Cystamine treatment is neuroprotective in the YAC128 mouse model of Huntington disease. J Neurochem. 95, 210-20.

Van Raamsdonk, J.M., Pearson, J., Rogers, D.A., Lu, G., Barakauskas, V.E., Barr, A.M., Honer, W.G., Hayden, M.R., Leavitt, B.R., 2005b. Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Experimental Neurology. 196, 266-72.

Van Raamsdonk, J.M., Pearson, J., Slow, E.J., Hossain, S.M., Leavitt, B.R., Hayden, M.R., 2005c. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci. 25, 4169-80.

von Hörsten, S., Schmitt, I., Nguyen, H.P., Holzmann, C., Schmidt, T., Walther, T., Bader, M., Pabst, R., Kobbe, P., Krotova, J., Stiller, D., Kask, A., Vaarmann, A., Rathke-Hartlieb, S., Schulz, J.B., Grasshoff, U., Bauer, I., Vieira-Saecker, A.M.M., Paul, M., Jones, L., Lindenberg, K.S., Landwehrmeyer, B., Bauer, A., Li, X.-J., Riess, O., 2003. Transgenic rat model of Huntington's disease. Hum Mol Genet. 12, 617-24.

Warby, S.C., Doty, C.N., Graham, R.K., Carroll, J.B., Yang, Y.-Z., Singaraja, R.R., Overall, C.M., Hayden, M.R., 2008. Activated caspase-6 and caspase-6-cleaved fragments of huntingtin specifically colocalize in the nucleus. Hum Mol Genet. 17, 2390-404.

Weigell-Weber, M., Schmid, W., Spiegel, R., 1996. Psychiatric symptoms and CAG expansion in Huntington's disease. Am J Med Genet. 67, 53-7.

Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology. 134, 319-29.

Wong, A.A., Brown, R.E., 2006. Visual detection, pattern discrimination and visual acuity in 14 strains of mice. Genes Brain Behav. 5, 389-403.

Wood, N.I., Goodman, A.O.G., van der Burg, J.M.M., Gazeau, V., Brundin, P., Björkqvist, M., Petersén, A., Tabrizi, S.J., Barker, R.A., Morton, A.J., 2008. Increased thirst and drinking in Huntington's disease and the R6/2 mouse. Brain Res Bull. 76, 70-9.

Young, A.B., Greenamyre, J.T., Hollingsworth, Z., Albin, R., D'Amato, C., Shoulson, I., Penney, J.B., 1988. NMDA receptor losses in putamen from patients with Huntington's disease. Science. 241, 981-3.

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, 493-7.

Zethof, T.J., Van der Heyden, J.A., Tolboom, J.T., Olivier, B., 1994. Stress-induced hyperthermia in mice: a methodological study. Physiol Behav. 55, 109-15.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B.R., Goffredo, D., Conti, L., MacDonald, M.E., Friedlander, R.M., Silani, V., Hayden, M.R., Timmusk, T., Sipione, S., Cattaneo, E., 2001. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science. 293, 493-8.

7. Discussion and future directions

The studies performed as part of this thesis employed a number of cellular, behavioural, and neuropathological measures in the YAC128 mouse model of HD to investigate the modulation of enhanced susceptibility to excitotoxic stress as a therapeutic approach for HD.

7.1. Excitotoxicity as a therapeutic target in HD

Enhanced susceptibility to excitotoxicity has long been suspected to contribute to the pathogenesis of HD, and evidence from cellular and animal models of HD continues to accumulate in support of this hypothesis. Although a number of trials with anti-excitotoxic agents have failed to show benefit in HD patients, these trials generally suffered from several limitations. First, whereas the evidence points to enhanced susceptibility to excitotoxic stress being an early event preceding symptomatic manifestation of the disease, all of the trials conducted to date were carried out in clearly symptomatic HD patients in whom the continued contribution of enhanced excitotoxicity to the disease phenotype is unclear. Second, whereas the evidence suggests that a long duration of treatment would be required for beneficial effects to be observed, the duration of treatment in many of the trials conducted to date has been relatively short. Third, the level of inhibition particularly as it relates to NMDA receptors is a critical factor, with very low doses of NMDA receptor antagonists being potentially of no benefit due to inadequate levels of inhibition, and high doses of NMDA receptor antagonists being of either no benefit or even detrimental due to excessive inhibition of NMDA receptor activity.

In light of these considerations, the use of rodent models of HD, such as YAC128 HD animals, has allowed for better interrogation of the potential role of excitotoxicity in HD and its amenability to therapeutic intervention. Specifically, it has allowed us to address whether early

and long-term treatment of YAC128 HD animals with compounds that target excitotoxicity can ameliorate the motor and neuropathological symptoms, and to examine dosage effects of compounds.

Furthermore, the use of the YAC128 HD mice has allowed us to address whether the different cellular pathways implicated in excitotoxic cell death are equally useful as targets for ameliorating the behavioural and neuropathological deficits of YAC128 HD mice. Moreover, the use of YAC128 HD mice has allowed us to examine whether a treatment that combines compounds targeting different cellular pathways can provide additional benefit than see by targeting a single pathway alone.

While these studies provide considerable novel information on the relative contribution of select cellular pathways and the potential utility of compounds targeting these pathways as treatment in HD, much remains to be explored. For example, would the compounds examined in this study result in similar effects in other rodent models of HD? Examining the effects of compounds that provided beneficial outcome in the present studies, such as memantine, in other mouse or rat models of HD would inform on the robustness of the findings and provide further support for their potential beneficial effects in HD. Also, continuing studies examining the pathogenic mechanisms contributing to the disease will help provide a better understanding of the mechanism of action of the compounds employed as well as result in additional candidate targets of potential therapeutic relevance.

7.1.1. Therapeutic targets in HD: NMDA receptors

Our trial of memantine in the YAC128 HD mice suggests that enhanced susceptibility to excitotoxic stress as mediated by excessive NMDA receptor activity is likely to contribute to HD

and that early therapeutic intervention may be able to ameliorate the disease phenotype, with both improvements in motor function and prevention of neuropathological deficits.

That treatment with memantine led to improvements in motor function and rescue of striatal pathology in the YAC128 HD animals is unique. Indeed, of 20 compounds tested thus far in YAC128 HD mice, only memantine has been found to result in late improve of motor dysfunction and rescue of neuropathological deficits. Of the compounds tested to date, treatment with cystamine, an inhibitor transglutaminase activity, prevented the striatal neuropathology but failed to improve motor function (Van Raamsdonk et al., 2005a), whereas treatment with Ethyl-EPA, a precursor of the omega-3 fatty acid EPA with presumed neuroprotective properties, improved motor function but failed to rescue striatal pathology (Van Raamsdonk et al., 2005b) in YAC128 HD mice. All other compounds tested failed to improve motor function or striatal neuropathology. These findings are encouraging and point to the potential use of memantine as a treatment in HD.

Evaluation of memantine in other animal models of HD would inform on the robustness of the findings in the present study. Indeed, demonstration of improvements in motor function and rescue of striatal pathology in other rodent models of HD expressing HD transgenes with different CAG sizes and with different transgene integration loci would provide further evidence of the potential beneficial effects of memantine in HD.

Unlike the trial of arimoclomol in YAC128 HD mice, in this study surrogates of target engagement were examined prior to the initiation of the long-term trial. Protection against NMDA receptor-mediated cell death by memantine treatment in MSNs ex vivo as well as in acute striatal lesioning studies in vivo was demonstrated, supporting inhibition of NMDA receptor by memantine in treated animals. Although direct demonstration of target engagement

in vivo, such as may be accomplished with PET ligand studies for example, would be ideal, in the absence of such a possibility the use of surrogate markers of target engagement may be adequate.

In this study, treatment with memantine was started at 2 months of age, before the onset of motor dysfunction. Recent studies in YAC128 HD mice suggest that enhanced susceptibility to excitotoxic stress is an early phenomenon, with its contribution to disease likely becoming less prominent with disease progression (Graham et al., 2009). As such, treatments aimed at ameliorating the enhanced susceptibility to excitotoxic stress are likely to have a more significant impact on disease phenotype when administered early in the disease, with treatment at the symptomatic stage likely becoming of diminishing value. To assess this possibility, future studies may examine the effect of treatment with memantine of symptomatic animals at more advance ages than used in the current studies (i.e. start of treatment at 6-7 months of age compared to the 2 months of age start point used in this study).

The present study of memantine also highlights the importance of dosage as a parameter requiring particular consideration in advancing memantine as a treatment for HD. Indeed, whereas low-dose memantine, which predominantly blocks extrasynaptic NMDA receptor activity, is protective, a high-dose of memantine, which blocks both synaptic and extrasynaptic NMDA receptors, had no effect on motor function and led to worsening of the striatal pathology in YAC128 HD mice. Establishing a surrogate marker that allows for the gauging of the extent of inhibition of synaptic versus extrasynaptic NMDA receptors will, therefore, be of great value in guiding dosage selection and the development of memantine as a treatment for HD patients.

7.1.2. Therapeutic targets in HD: mitochondrial viability/function

We evaluated the therapeutic potential of rasagiline in the YAC128 HD mice. Our choice of rasagiline was based on demonstrations of its neuroprotective properties in the face of neuronal stress paradigms, particularly, its effects in stabilizing the mitochondrial membrane potential and its prevention of the induction of mitochondrial permeability transition pore in response to cellular insults. Similar to our study with memantine, prior to the initiation of the long-term treatment trial in YAC128 HD animals, we first demonstrated that treatment with rasagiline protects against neurotoxicity in cultured MSNs ex vivo, and is neuroprotective in acute striatal lesioning models in vivo. These studies served as surrogates of rasagiline's likely ability to protect against the enhanced susceptibility to excitotoxic stress in YAC128 HD mice.

Although rasagiline has MAOB inhibitory activity, its neuroprotective properties are largely attributed to its propargyl moiety and are thought to be independent of its inhibition of MAOB. This conclusion is based on studies examining its optical isomer TV-1022, which exhibits 1000x less MAOB inhibitory activity, yet possesses neuroprotective properties equivalent to those of rasagiline. Whether the improvements observed following rasagiline treatment in YAC128 HD are indeed independent of MAOB inhibition remains to be determined, and future studies may address this at least partly by examining the effects of TV-1022 in YAC128 HD animals.

Furthermore, while numerous studies have demonstrated the effects of rasagiline treatment on mitochondrial function and its response to stress, these effects have not been assessed directly in the YAC128 HD mice as part of this study. Verification that the presumed targets of rasagiline action, such as Bcl-2, FAS, and Bcl-xL expression, PKC activation, and reduced mPT induction following stress have indeed been engaged in MSNs of treated YAC128 HD animals will be important as part of future follow-up studies.

Also, a more detailed examination of rasagiline dosage effects may be warranted. Indeed, while treatment with a dose of 0.1 mg/kg of rasagiline resulted in clear improvements in motor function in YAC128 HD mice, a dose of 1 mg/kg of rasagiline resulted in no improvements in YAC128 HD mice, with a trend toward potentially detrimental effects in WT animals. Thus, evaluation of doses lower than 0.1 mg/kg may help inform on the minimum efficacious dose in YAC128 HD animals.

Finally, whether the benefit conferred by rasagiline treatment on motor function reflects disease modifying or simply symptomatic effects awaits further examination. Assessment of motor function following a washout period that would allow clearance of the compound from the body would be informative in this regard. Continued improvements in motor function following washout would support that the improved motor function in rasagiline-treated animals reflects disease-modifying effects. Similarly, future studies may examine whether treatment with rasagiline after onset of motor dysfunction (e.g. start of treatment at 6-7 months of age) can lead to slowing or reversal of disease progression.

7.1.3. Therapeutic targets in HD: combination therapy

To date, a range of mutant htt-mediated molecular disturbances have been identified in HD. Despite considerable evidence implicating excitotoxicity in HD, its primacy as a target remains uncertain. The relative importance of the different excitotoxicity-related cellular targets is similarly unclear. Given the multiplicity of targets implicated in HD, and the challenge in determining their relative contribution to disease pathogenesis, the likelihood that modulation of any single target is going to yield marked or complete amelioration of the symptoms of HD is small. Targeting of multiple pathways implicated in HD using combination therapy may be helpful in this regard.

Our findings from the memantine and rasagiline combination study serve to support this proposition. Whereas treatment with memantine ameliorated the striatal neuropathology and resulted in motor function improvements late in the disease phenotype, and treatment with rasagiline resulted in early improvements in motor function but no effect on striatal neuropathology, treatment with memantine and rasagiline in combination led to early and sustained improvements in motor function and rescue of striatal pathology in the YAC128 HD.

7.1.4. Therapeutic targets in HD: heat shock proteins

Increased expression, and therefore activity, of heat shock proteins, such as HSP-70, has been shown to protect against excitotoxicity (Dedeoglu et al., 2002) and to be neuroprotective in different models of polyglutamine-mediated toxicity (Fujikake et al., 2008; Fujimoto et al., 2005; Katsuno et al., 2005; Perrin et al., 2007; Vacher et al., 2005; Zhang and Sarge, 2007). We hypothesized that increase heat shock protein expression would similarly combat excitotoxic stress and ameliorate the phenotype in the YAC128 HD mice. Our trial of arimoclomol, a compound shown previously to increase the expression of heat shock proteins by enhancing the activation of the heat shock protein-inducing factor Hsf-1, has been inconclusive in evaluating this hypothesis in the YAC128 HD mice. Acute and sub-chronic treatment with arimoclomol failed to protect against QA-mediated excitotoxic striatal lesioning. Furthermore chronic treatment with arimoclomol did not improve the motor function or rescue striatal pathology in YAC128 HD mice. That arimoclomol failed to improve the phenotype of YAC128 HD mice likely reflects the lack of expected induction of heat shock proteins following arimoclomol treatment. This may, in turn, be the result of the different dose used (80 mg/kg in this study vs. 10 mg/kg in other rodent studies (Kalmar et al., 2008; Kieran et al., 2004)), the different route of

administration (p.o. in this study vs. i.p. other rodent studies (Kalmar et al., 2008; Kieran et al., 2004)), or both.

The lack of up-regulation of a heat shock protein response, as represented by a lack of increase in Hsp90 and Hsp70 expression following arimoclomol treatment in YAC128 HD mice, represents a major caveat of this study. Indeed, the potential therapeutic benefit of up-regulating the heat shock protein response in HD remains unclear. Future studies with arimoclomol specifically and heat shock protein inducers in general will be well served by setting heat shock protein induction following short-term treatment as a requirement for continued long-term evaluation in mouse models of HD. Finally, compounds such as geranylgeranylacetone (GGA), a nontoxic antiulcer drug, have been shown to potently up-regulate expression of heat shock proteins including Hsp70, Hsp90, and Hsp105 in various tissues, including the CNS (Katsuno et al., 2005), and are potential candidates as alternative heat shock response inducers for trial in mouse models of HD.

7.1.5. Therapeutic targets in HD: metabotropic glutamate receptors

Group I metabotropic glutamate receptor 5 (mGluR5) represents a potential excitotoxicity-related target for therapeutic intervention. Several lines of evidence support this proposition. First, mGluR5 receptors are preferentially expressed in the striatal projection neurons selectively lost in HD compared to the spared interneurons (Kerner et al., 1997; Tallaksen-Greene et al., 1998). Second, mGluR5 receptor activation results in marked enhancement of NMDA-induced membrane depolarization and intracellular Ca^{2+} accumulation in striatal neurons (Calabresi et al., 1999; Pisani et al., 2001), an effect that is prevented by treatment with the mGluR5 antagonist MPEP. Importantly, the enhancement in NMDA-induced currents is not observed in the large aspiny interneurons spared in HD (Calabresi et al., 1999). Finally, inhibition of mGluR5 signaling using selective non-competitive antagonists of mGluR5 is neuroprotective against

NMDA-mediated excitotoxicity both in neuronal cultures in vitro and in acute striatal excitotoxicity paradigms in vivo (e.g. QA-mediated striatal lesioning) (Bruno et al., 2000; Orlando et al., 2001; Popoli et al., 2004).

Furthermore, mGluR5 receptors are known to contribute to increased cytosolic Ca^{2+} levels and dysregulated intracellular calcium homeostasis through phospholipase C (PLC)-mediated generation of $InsP_3$ and the activation of ER-bound $InsP_3R_1$ receptors. Mutant htt has been shown to sensitize $InsP_3R_1$ receptor to activation by $InsP_3$ and facilitate ER Ca^{2+} release in response to sub-threshold concentrations of mGluR5 agonists, effects that lead to enhanced glutamate-induced excitotoxicity and which are not observed with wildtype htt (Tang et al., 2003). Moreover, treatment of MSNs with the $InsP_3R_1$ blockers 2-APB and enoxaparin conferred protection against glutamate-induced excitotoxicity.

Although beyond the scope of the studies constituting this thesis, these observations nominate mGluR5 receptors as worthy of further investigation as an excitotoxicity-related therapeutic target in HD.

7.1.6. Therapeutic targets in HD: glutamate reuptake/glutamate transporters

Removal of extracellular glutamate following its release is critical to the maintenance of its physiological levels and to appropriate signaling through its receptors. The glutamate transporters GLT-1 (glutamate transporter-1) and GLAST (glutamate aspartate transporter), expressed in astrocytes, are the primary mediators of glutamate removal, and disturbances in their functioning may contribute to enhanced excitotoxicity and neuronal degeneration. Indeed, disturbances in glutamate transport have been implicated in a number of degenerative disorders. Expression of ataxin-7, the polyglutamine-containing protein underlying spinocerebellar ataxia type 7 (SCA7) disorder in cerebellar Bergmann glial cells results in significant decreases in

mRNA and protein levels of GLAST, an effect that likely contributes to the disease pathology (Custer et al., 2006). Similarly, striatal and cortical expression of GLT-1 mRNA expression is significantly decreased in R6/2 animals expressing exon 1 of mutant htt, an effect that is associated with decrease glutamate uptake and is likely to contribute to disease pathogenesis (Behrens et al., 2002; Estrada-Sánchez et al., 2009). Similar down-regulation of astrocytic GLT-1 expression has also been observed in tissues from ALS patients (Rothstein et al., 1995) and in animal models of ALS (Bruijn et al., 1997).

It is, therefore, possible that enhancing the removal of glutamate by increasing the expression or activity of glutamate transporters may mitigate the increased susceptibility to excitotoxic stress that results from excessive NMDA receptor activation in HD. This approach has been shown to be possible in a model of ischemic injury in vitro as well as in an animal model of ALS, where treatment with the β-lactam ceftriaxone resulted in increased expression of GLT-1 and conferred neuroprotection (Rothstein et al., 2005). Thus, evaluation of strategies aimed at enhancing glutamate re-uptake by up-regulating the expression or activity of GLT-1 or GLAST as a therapeutic approach in HD is worthy of consideration for future studies.

7.2. Considerations for clinical trials in HD

The preclinical trials conducted as part of this thesis were designed to assess neuroprotection and modification of the underlying disease process. The use of inbred mouse strains and the availability of robust, progressive, and longitudinal behavioural and biochemical endpoints makes the assessment of any potential "disease-modifying" effects of such compounds in rodents possible. However, validating such disease-modifying effects in HD patients poses particular challenges.

Patient population. Neuroprotective compounds are generally thought to provide the greatest benefit when acting very early on in the disease process, well before the onset of profound symptoms. As such, the choice of an appropriate population of HD gene carriers to treat in such a trial becomes critical (i.e pre-manifest, close to onset, early symptomatic, mid-stage symptomatic, or late stage HD?). In the case of pre-manifest HD gene carriers, the slow progressive nature of the disease has made the development of longitudinal measures that demonstrate progression over a relatively short period of time (i.e. 1 year period) difficult. This difficulty is further exacerbated by the heterogeneity in disease progression that relates to differences in CAG repeat size between subjects (Aziz et al., 2009). Given the dearth of such measures, efforts are underway to establish sensitive and reliable biomarkers for longitudinal detection of progression in pre-manifest and early HD (Tabrizi et al., 2009).

Duration of treatment. Given the slow progression of the disease, another consideration in conducting a clinical trial in HD is the duration of treatment. Trial designs that incorporate extended treatment durations may be necessary for significant improvement and slowing of disease progression to be observed.

Choice of endpoints. The choice of primary and secondary endpoints is an important factor and will need to reflect the mode of action of the compound under investigation as well as the expected outcome. For example, NMDA receptor activity is critical to learning and memory and compounds that target NMDA receptor activity, such as memantine, have been shown to influence cognition in human dementia trials. Incorporation of cognitive function measures may, therefore, be appropriate for the assessment of such compounds in HD. Additional endpoints that may provide an objective assessment protective effects are brain imaging biomarkers. For such modalities, the choice of imaging approaches that provide structural measures, such as brain

volumetric changes assessed by magnetic resonance imaging, compared to those that provide functional measures, such as levels of neuronal metabolites by magnetic resonance spectroscopy, will need further consideration.

Statistical considerations. The slow progression of the disease, coupled with the heterogeneity in the rates of progression between patients with different CAG sizes, makes the expected effect sizes modest and the likely variability large. As such, appropriate statistical considerations in the design of trials and adequate powering will become crucial for detection of treatment effects.

Dosage and verification of target engagement. As illustrated by the memantine study in YAC128 HD animals, appropriate dosage is an important consideration in conducting a meaningful trial in HD patients. The translation of dosage information from rodents to patients in HD is an area that needs further exploration. In this context, the development of surrogate markers for in vivo engagement of the target of interest becomes not only important for validation of its involvement, but may also help in gauging the appropriate dosage.

7.3. Depressive behaviour in YAC128 HD animals

Our findings from the study of depressive-like phenotype in YAC128 HD animals suggests that the depressive symptoms in HD patients is likely to be, at least in part, the result of mutant htt-related neurobiological effects and not simply a reflection of the psychosocial milieu of HD patients. Consistent with observations in HD patients with depression, the depressive phenotype in YAC transgenic animals expressing mutant htt is both age- and CAG size-independent.

The FST as a test of depressive behaviour has been employed routinely in the past in the screening of compounds with antidepressant activities, as it is readily amenable to automation with computer-based video scoring of behaviour (Crowley et al., 2004; Petit-Demouliere et al., 2005). Given that the depressive phenotype is detected in YAC transgenic animals as early as 3

months of age using the FST, the FST in YAC128 HD mice may be used to screen for anti-depressant compounds that may be of benefit for depression in HD.

In our study, treatment with a selective serotonin reuptake inhibitor, fluoxetine, or a tricyclic antidepressant, imipramine, failed to improve the depressive symptoms of YAC128 HD animals. This mirrors the considerable variability in responsiveness to anti-depressant treatment in HD patients (Leroi and Michalon, 1998). To date, no definitive trial of antidepressant treatment in HD has been conducted, and efforts to ameliorate the depressive symptoms in HD would benefit from such trials.

7.3.1. Development of additional endpoints for trials in HD

Motor deficits and psychiatric disturbances are prominent features of HD, and clear and robust endpoints have been developed to assess these features in YAC128 mouse model of HD. However, a primary complaint of HD patients relates to cognitive dysfunction, and further development of robust assays that provide reliable assessment of cognitive function in YAC128 HD mice will be of great importance in identifying potential pharmacological interventions that may benefit this domain of function.

Although numerous assays of cognitive function in rodents have been developed to date, a key consideration in selecting ones for examination in the YAC128 HD mice is suitability for use in drug screening. Two such assays that allow for higher thoughput testing are the novel object recognition and trace fear conditioning tests of cognitive function. Fear conditioning is a test that has been widely applied to measure an animal's ability to learn and associate neutral cue or context and an aversive stimulus, such as an electric shock. Deficits in fear conditioning may be indicative of cognitive dysfunction (Maren, 2001).

The novel object recognition assay is a one-trial test of memory that utilizes a rodent's innate tendency to explore novel objects in their environment (Ennaceur and Delacour, 1988). In this task, the animals are first presented with two identical objects and allowed to explore them freely. One of the objects is then switched and the amount of time the animal spends exploring the objects in this second testing session. Normal animals will preferentially explore the novel object, indicative of memory for the familiar object, whereas animals with memory deficits will do so to a lesser extent.

Given the known differences in the amenability of different mouse strains to tests of cognitive function, determining the optimal background strain for cognitive tests of most relevance to HD (e.g. corticostriatally-dependent functions) will be of utmost importance.

7.4. Conclusions

The studies performed as part of this thesis support the targeting of excitotoxicity, and in particular extrasynaptic NMDA receptors, as a potential therapeutic approach for the treatment of HD. Indeed, an international effort is currently underway to consider the trial of memantine as a therapy for HD. The findings warrant further studies into the excitotoxic process and improved approaches to combat it as a therapeutic strategy in HD.

7.5. References

Aziz, N., Jurgens, C., Landwehrmeyer, G., Group, o.b.o.t.E.R.S., van Roon-Mom, W., van Ommen, G., Stijnen, T., Roos, R., 2009. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. Neurology.

Behrens, P.F., Franz, P., Woodman, B., Lindenberg, K.S., Landwehrmeyer, G.B., 2002. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain. 125, 1908-22.

Bruijn, L.I., Becher, M.W., Lee, M.K., Anderson, K.L., Jenkins, N.A., Copeland, N.G., Sisodia, S.S., Rothstein, J.D., Borchelt, D.R., Price, D.L., Cleveland, D.W., 1997. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron. 18, 327-38.

Bruno, V., Ksiazek, I., Battaglia, G., Lukic, S., Leonhardt, T., Sauer, D., Gasparini, F., Kuhn, R., Nicoletti, F., Flor, P.J., 2000. Selective blockade of metabotropic glutamate receptor subtype 5 is neuroprotective. Neuropharmacology. 39, 2223-30.

Calabresi, P., Centonze, D., Pisani, A., Bernardi, G., 1999. Metabotropic glutamate receptors and cell-type-specific vulnerability in the striatum: implication for ischemia and Huntington's disease. Experimental Neurology. 158, 97-108.

Custer, S.K., Garden, G.A., Gill, N., Rueb, U., Libby, R.T., Schultz, C., Guyenet, S.J., Deller, T., Westrum, L.E., Sopher, B.L., La Spada, A.R., 2006. Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. Nat. Neurosci. 9, 1302-11.

Dedeoglu, A., Ferrante, R.J., Andreassen, O.A., Dillmann, W.H., Beal, M.F., 2002. Mice overexpressing 70-kDa heat shock protein show increased resistance to malonate and 3-nitropropionic acid. Experimental Neurology. 176, 262-5.

Estrada-Sánchez, A.M., Montiel, T., Segovia, J., Massieu, L., 2009. Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. Neurobiology of Disease. 34, 78-86.

Fujikake, N., Nagai, Y., Popiel, H.A., Okamoto, Y., Yamaguchi, M., Toda, T., 2008. Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem. 283, 26188-97.

Fujimoto, M., Takaki, E., Hayashi, T., Kitaura, Y., Tanaka, Y., Inouye, S., Nakai, A., 2005. Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. J Biol Chem. 280, 34908-16.

Graham, R.K., Pouladi, M.A., Joshi, P., Lu, G., Deng, Y., Wu, N.-P., Figueroa, B.E., Metzler, M., André, V.M., Slow, E.J., Raymond, L., Friedlander, R., Levine, M.S., Leavitt, B.R., Hayden, M.R., 2009. Differential susceptibility to excitotoxic stress in YAC128 mouse

models of Huntington disease between initiation and progression of disease. J Neurosci. 29, 2193-204.

Kalmar, B., Novoselov, S., Gray, A., Cheetham, M.E., Margulis, B., Greensmith, L., 2008. Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. J Neurochem. 107, 339-50.

Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., Doyu, M., Sobue, G., 2005. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci USA. 102, 16801-6.

Kerner, J.A., Standaert, D.G., Penney, J.B., Young, A.B., Landwehrmeyer, G.B., 1997. Expression of group one metabotropic glutamate receptor subunit mRNAs in neurochemically identified neurons in the rat neostriatum, neocortex, and hippocampus. Brain Res Mol Brain Res. 48, 259-69.

Kieran, D., Kalmar, B., Dick, J.R.T., Riddoch-Contreras, J., Burnstock, G., Greensmith, L., 2004. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med. 10, 402-5.

Orlando, L.R., Alsdorf, S.A., Penney, J.B., Young, A.B., 2001. The role of group I and group II metabotropic glutamate receptors in modulation of striatal NMDA and quinolinic acid toxicity. Experimental Neurology. 167, 196-204.

Perrin, V., Régulier, E., Abbas-Terki, T., Hassig, R., Brouillet, E., Aebischer, P., Luthi-Carter, R., Déglon, N., 2007. Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. Mol Ther. 15, 903-11.

Pisani, A., Gubellini, P., Bonsi, P., Conquet, F., Picconi, B., Centonze, D., Bernardi, G., Calabresi, P., 2001. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience. 106, 579-87.

Popoli, P., Pintor, A., Tebano, M.T., Frank, C., Pepponi, R., Nazzicone, V., Grieco, R., Pèzzola, A., Reggio, R., Minghetti, L., De Berardinis, M.A., Martire, A., Potenza, R.L., Domenici, M.R., Massotti, M., 2004. Neuroprotective effects of the mGlu5R antagonist MPEP towards quinolinic acid-induced striatal toxicity: involvement of pre- and post-synaptic mechanisms and lack of direct NMDA blocking activity. J Neurochem. 89, 1479-89.

Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J., Kuncl, R.W., 1995. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol. 38, 73-84.

Rothstein, J.D., Patel, S., Regan, M.R., Haenggeli, C., Huang, Y.H., Bergles, D.E., Jin, L., Dykes Hoberg, M., Vidensky, S., Chung, D.S., Toan, S.V., Bruijn, L.I., Su, Z.-Z., Gupta, P., Fisher, P.B., 2005. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 433, 73-7.

Tabrizi, S., Langbehn, D., Leavitt, B., Roos, R., Durr, A., Craufurd, D., Kennard, C., Hicks, S., Fox, N., Scahill, R., Borowsky, B., Tobin, A., Rosas, H., Johnson, H., Reilmann, R., Landwehrmeyer, B., Stout, J., investigators, t.T.-H., 2009. Biological and clinical

manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet neurology.

Tallaksen-Greene, S.J., Kaatz, K.W., Romano, C., Albin, R.L., 1998. Localization of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified populations of striatal neurons. Brain Res. 780, 210-7.

Tang, T.-S., Tu, H., Chan, E.Y.W., Maximov, A., Wang, Z., Wellington, C.L., Hayden, M.R., Bezprozvanny, I., 2003. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. Neuron. 39, 227-39.

Vacher, C., Garcia-Oroz, L., Rubinsztein, D.C., 2005. Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. Hum Mol Genet. 14, 3425-33.

Van Raamsdonk, J.M., Pearson, J., Bailey, C.D.C., Rogers, D.A., Johnson, G.V.W., Hayden, M.R., Leavitt, B.R., 2005a. Cystamine treatment is neuroprotective in the YAC128 mouse model of Huntington disease. J Neurochem. 95, 210-20.

Van Raamsdonk, J.M., Pearson, J., Rogers, D.A., Lu, G., Barakauskas, V.E., Barr, A.M., Honer, W.G., Hayden, M.R., Leavitt, B.R., 2005b. Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Experimental Neurology. 196, 266-72.

Zhang, Y.-Q., Sarge, K.D., 2007. Celastrol inhibits polyglutamine aggregation and toxicity though induction of the heat shock response. J Mol Med. 85, 1421-8.

Appendix: Animal Care Certificate

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A07-0106

Investigator or Course Director: Michael Hayden

Department: Medical Genetics

Animals:

Mice FVB 9000
Mice C57Bl/6 420
Mice 129 100

Start Date: **March 15, 2007** **Approval Date:** **July 20, 2007**

Funding Sources:

Funding Agency:	High Q Foundation
Funding Title:	TREAT-HD: Translational Research on Excitotoxicity to Accelerate Therapies for Huntington's Disease (SPOC Studies - HTT Phosphorylation)
Funding Agency:	High Q Foundation
Funding Title:	TREAT-HD: Translational Research on Excitotoxicity to Accelerate Therapies for Huntington's Disease (Scientific Proof of Concept Studies)
Funding Agency:	Aspreva Pharmaceuticals SA
Funding Title:	SA: Head-to-head comparison of CellCept and Zocor for Atherosclerosis prevention

Funding Agency:	High Q Foundation
Funding Title:	HDSA Coalition for the Cure / TREAT HD
Funding Agency:	Canadian Institutes of Health Research (CIHR)
Funding Title:	Regulation of Huntington Phosphorylation by Akt and BDNF and its Role in Huntington Disease
Funding Agency:	Vertex Pharmaceuticals Inc.
Funding Title:	Efficacy of caspase inhibitors in reducing excitotoxic neural cell death precipitated by mutant huntingtin"
Funding Agency:	Huntington Society of Canada
Funding Title:	Phosphorylation of Hungtington on Serine 421 (pS421) by AKT: its role in the pathogenesis of HD
Funding Agency:	Networks of Centres of Excellence (NCE)
Funding Title:	Triplet Repeat Mutations: Huntington Disease
Funding Agency:	Jack and Doris Brown Foundation
Funding Title:	Genetics of Neurodegeneration
Funding Agency:	Canadian Institutes of Health Research (CIHR)
Funding Title:	Regulation of body weight: Hypothalamic mediators and their peripheral targets
Funding Agency:	Unfunded Research
Funding Title:	Animal Model of Huntington's Disease
Funding Agency:	Donations for Health Science Research
Funding Title:	Family Donations
Funding Agency:	Merck Frosst Canada Inc.
Funding Title:	Genetics of Apoptosis and Neurodegeneration

Funding Agency:	Huntington's Disease Society of America
Funding Title:	Proteolytic cleavage and the pathogenesis of Huntington's Disease
Funding Agency:	Canadian Institutes of Health Research (CIHR)
Funding Title:	The development and use of YAC transgenic mice to explore the pathogenesis of Huntington disease
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Neuroprotective Roles of Wild Type Huntington
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Assessment of in vivo caspase inhibition on the pathogenesis of Huntington disease in YAC transgenic mice
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Proteolytic Cleavage and the Pathogenesis of Huntingtons Disease
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Huntingtin Function
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Neuroprotective role of wild-type Huntingtin
Funding Agency:	High Q Foundation
Funding Title:	TREAT-HD: Translational Research on Excitotoxicity to Accelerate Therapies for Huntington's Disease
Funding Agency:	Canadian Institutes of Health Research (CIHR)
Funding Title:	Phosphorylation of Hungtington on Serine 421 (pS421) by AKT: its role in the pathogenesis of HD
Funding Agency:	British Columbia Research Institute for Children and Women's Health
Funding Title:	The role and modulation of glutamate regulated (mGluR5/InsP3R1) huntington toxicity in Huntington's Disease

Funding Agency:	Michael Smith Foundation for Health Research
Funding Title:	FIND: Fundamental innovation in neurodegenerative diseases
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Neuroprotective roles of wild type Huntington
Funding Agency:	Huntington's Disease Society of America
Funding Title:	Huntingtin Proteolysis and Posttranslational Modification
Funding Agency:	High Q Foundation
Funding Title:	HDSA Coalition for the Cure / TREAT HD
Unfunded title:	n/a

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3
Phone: 604-827-5111 Fax: 604-822-5093