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The role of trophic factors and other drugs in the treatment of Huntington's disease in R6/2 mouse model

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The Role of Trophic Factors and Other Drugs in the Treatment of Huntington's Disease in R6/2 Mouse Model

by

Jessica Ciesler

Submitted to the Graduate Faculty as a partial fulfillment of the requirement for the Master of Science Degree in Pharmacology and Toxicology

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An Abstract of

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Huntington's disease (HD) is a rare, autosomal dominant, neurodegenerative disease, characterized by behavioral and cognitive deterioration, as well as a progressive loss in motor control. HD is thought to be caused by the expansion of trinucleotide, CAG, expansion at the N-terminus within exon 1 of the huntingtin gene. The huntingtin protein is considered to play a role in the cell survival and apoptosis pathways of neurons, including Akt kinase and JNK. We hypothesized that the treatment of the R6/2 transgenic mouse model with neurotrophic peptides, Colivelin and analogue dPEG-Colivelin, Sulforaphane, a compound that reduces oxidative stress, and Ceftriaxone, a GLT-1 upregulator, would attenuate motor behavioral abnormalities and neurodegeneration in HD R6/2 mouse model.

Although we did not observe any behavioral motor improvement with Colivelin and dPEG-Colivelin, we did, in fact, find significant up-regulation of *p*-Akt in the ST of 40 mg/20g Colivelin treated R6/2 mice as compared to saline treated R6/2 mice. However, we did not observe any significant difference in the level of *p*-Akt in the PFC with Colivelin and dPEG-Colivelin treatments as compared to saline treated groups. Moreover, significant increase in *p*-JNK was observed in the ST and PFC of 40 mg/20g Colivelin treated R6/2 mice. Alternatively, we did not observe any behavioral improvements with Ceftriaxone or Sulforaphane.

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Chapter 1

Introduction

1.1 Huntington's Disease Overview

Huntington's disease (HD) is a rare, autosomal dominant, neurodegenerative disease that was first accurately described by 22 year old American doctor, George Huntington, in 1872 (Bonelli and Beal 2012). Huntington described a noticeable hereditary chorea that led to suicide and insanity, which manifested in adulthood, ultimately leading to death (Huntington 2003). Huntington was not the first to acknowledge this disorder. Chorea, or dancing mania, was described as early as 1374, and it was Paracelsus who first coined the word *chorea*. It has been reported that at least one of the alleged witches of the Salem Witch Trials (Massachusetts 1690s) had HD, as many people in those times thought the involuntary muscle jerks and twitches distinctive of HD were a result of being possessed by the devil. In the 1920s, Charles B. Davenport conducted the largest HD study of its time, tracking families with the disease. In the 1970s Nancy Wexler discovered the HD gene [for review see ref. (Zuccato, Valenza et al. 2010)]. According to a 2012 meta-analysis, HD occurs in 5.70 per 100,000 people in North America, Europe, and Australia (Pringsheim, Wiltshire et al. 2012). HD is

illustrated by behavioral and cognitive deterioration (Aiken, Tobin et al. 2004), as well as a progressive loss in motor control. Appearance of HD occurs around the age of 40, with cases that arise as early as infancy and as late as the age of 80, with an average duration of about 17 years (Bonelli and Beal 2012).

The characteristic chorea is the hallmark of the disease, consisting of abnormal involuntary movements across the body, as well as impairment of normal, voluntary movements (Frank and Jankovic 2010). These jerky, "cigarette flicking" movements are most commonly seen early in the disease in hands and facial muscles. As the disease progresses, the movements can turn into flailing of the limbs or slower, flowing movements. Progressive weight loss, changes in sexual behavior and sleep cycle are a few commonly reported symptoms early in the disease, thought to be related to dysfunction of the hypothalamus (Politis, Pavese et al. 2008). Motor symptoms occur alongside ongoing impairment of mental processes involved in reasoning, judgment, memory, and comprehension (Bates, Harper et al. 2002; Rosenblatt 2007). Patients become unable to walk, talk, maintain dietary and personal care needs, and the risk of falling, infection, and choking become an imminent threat in later stages of HD (Bates, Harper et al. 2002). Newly diagnosed patients can usually maintain independent living and employment, but further along the years early retirement and the surrendering of driving privileges become necessary. Managed care, or institutionalization, is needed once advanced motor loss, personality changes, and mood disturbances become unmanageable (Zuccato, Valenza et al. 2010). The massive neurodegeneration exhibits itself physically and mentally; behavioral changes are thought to be evident up to fifteen years prior to presentation of motor symptoms (Paulsen, Langbehn et al. 2008).

Neuronal death occurs in the cerebral cortex and in the striatum (Aiken, Tobin et al. 2004), with the most predominate effects and change occurring in the striatum, caudate nucleus, and putamen (Reiner, Albin et al. 1988; Vonsattel and DiFiglia 1998; Pillai, Hansen et al. 2012). Although the mechanism of neuronal death in HD is not well defined, apoptosis plays a critical role in this mechanism (Hickey and Chesselet 2003). There is clear evidence of atrophy in postmortem HD brains. Studies examined HD postmortem brains found about 30% reduction in brain weights as compared to non-HD individuals. The frontal lobes showed atrophy in 80% of HD patients, and the striatum showed loss in 95% of cases, with neuronal striatal loss indicating that the caudate nucleus might be gradually affecting other areas during the progression of the disease (Roth, Klempii et al. 2005). It has been found that not all regions of the brain are affected equally. For example, the medial temporal lobe, including the hippocampus is largely well conserved (de la Monte, Vonsattel et al. 1988; Halliday, McRitchie et al. 1998). Higher levels of functional capabilities could be helped by preservation of these areas despite degeneration of the striatum (Pillai, Hansen et al. 2012).

Neuronal death in HD may not be dependent on interneuronal connectivity in the brain; it may be cell-autonomous (Aiken et al., 2004). HD patients display death and degeneration of medium spiny neurons, resulting in symptomatic HD development. With the degeneration of GABAergic medium spiny neurons in the caudate and in the globus pallidus, a reduction in dopamine (D) D1 and D2- receptor binding sites have been detected in association with the progression of the disease (Hedreen and Folstein 1995; Turjanski, Weeks et al. 1995; Ginovart, Lundin et al. 1997). Frontal-lobe volumes are correlated with memory, planning performance, and other cognitive measures (Jernigan,

Salmon et al. 1991; Brandt, Bylsma et al. 1995; Backman, Robins-Wahlin et al. 1997). Presymptomatic and early manifestations of cognitive performance have also been associated with the thalamus, insula, and white matter (Beglinger, Nopoulos et al. 2005; Kassubek, Juengling et al. 2005; Peinemann, Schuller et al. 2005; Rosas, Tuch et al. 2006). It is noteworthy that endogenous regulation of neurotrophic factor expression may play a key role in neuroprotection of cortical and striatal neurons. Potent neuroprotective effects have been shown by neurotrophins and ciliary neurotrophic factors on different neuronal populations of the striatum (Alberch, Perez-Navarro et al. 2004).

1.2 Mutant Huntingtin Protein in Huntington's Disease:

1.2.1 Normal Function of Huntingtin

This disease is suggested to be caused by the expansion of a trinucleotide (CAG) repeat within exon 1 of the huntingtin gene on chromosome 4 (4p16.3), expanding the polyglutamate region at the N-terminus (Andrew, Goldberg et al. 1993). The mutation of normal huntingtin results in an expanded stretch of glutamine residues (Bonelli and Beal 2012). This expansion is autosomal dominant, resulting in a loss-of-function mutation (Rubinsztein 2006) as well as accumulation of the polyglutamine (polyQ) extended huntingtin protein fragments, which may be a key factor, as they build up in the cytoplasm and nucleus [for review see ref. (Sari 2011)]. These expanded polyQ regions make medium spiny neurons in the striatum more susceptible to cell death, as well as death and dysfunction of neurons in the cortex, and other brain regions (Reiner, Albin et al. 1988). The length of the polyQ repeats may determine the grade of impacted

pathology. When the number of CAG repeats exceeds 35, the mutated form of the huntingtin protein (mhtt) is expressed leading to HD. Those whose fall within 6 – 35 CAG repeats, will not develop HD, but those with 36 – 39 CAG repeats could display incomplete penetrance [for review see ref. (Sari 2011)]. It is has been proposed that the number of CAG repeats is associated with the age of disease onset. Thus, higher number of CAG repeats is inversely associated with earlier age of onset (Andrew, Goldberg et al. 1993; Aziz, Jurgens et al. 2009). Other genetic modifiers have also been associated with the age at onset of HD, including single nucleotide polymorphisms (SNP). SNPs are found in genes that encode for htt, as well as proteins involved in transcriptional regulation, neurotransmission (GluR6, A2A, NR receptors), intracellular transport and endocytosis (HAP1), autophagy (autophagy-related related gene, Atg7), proximal function (peroxisome proliferator-activated receptor C coactivator 1a, PPARGC1A), and proteolysis (Shang, Danek et al. 2012).

Normal huntingtin protein (htt) is ubiquitously expressed, although moderately, both in and outside of the CNS. Htt is a soluble protein comprising of 3,144 amino acids, essential for embryogenesis (Ferrante, Gutekunst et al. 1997; Cattaneo, Rigamonti et al. 2001). While the various locations of subcellular htt do not appear to simplify the definition of its true function, htt is associated with various cellular structures. These associations include the Golgi apparatus, endoplasmic reticulum, and the nucleus; as well as presence in neurites and at synapses where htt interacts with microtubules and other vesicular structures (DiFiglia, Sapp et al. 1995; Velier, Kim et al. 1998; Hilditch-Maguire, Trettel et al. 2000; Hoffner, Kahlem et al. 2002; Kegel, Meloni et al. 2002; Li, Plomann et al. 2003).

Although htt itself has no sequence homology with other proteins, the polyQ region is present in many transcription factors (Everett and Wood 2004). This proposes a physiological function wherein, the polyQ region acts as a binding regulator towards other transcription factors that also contain a polyQ region (Perutz, Johnson et al. 1994; Harjes and Wanker 2003; Goehler, Lalowski et al. 2004; Li and Li 2004). The functionally active carboxy-terminus of htt contains nuclear export signals and nuclear localization signals that may establish the ability of the protein to transport molecules from the nucleus to the cytoplasm; this is demonstrated by htt's perinuclear and nuclear distribution. It appears that the 17 amino acids before the polyQ region of htt interacts with nuclear pore protein translocated promoter region (TPR), exporting proteins from the nucleus, and removal of this region results in htt accumulation in the nucleus (Xia, Lee et al. 2003; Cornett, Cao et al. 2005).

The presence of htt is essential for embryonic development, especially in the formation of the CNS. Mouse models carrying a huntingtin-knockout resulted in embryonic death (Zeitlin, Liu et al. 1995), and mice with less than 50% of normal htt levels had embryotic malformations including defects in the neuronal tube, cortex, striatum, and reduced neurogenesis (White, Auerbach et al. 1997). Htt has shown to be necessary for neuronal identity establishment in the striatum and cortex as well (Reiner, Del Mar et al. 2001). Neuroprotection by normal htt does not appear to end with development. In vivo studies showed a gene-dosage effect against excitotoxicity, and enhanced neuroprotection as levels of normal htt are increased (Zhang, Li et al. 2003; Leavitt, van Raamsdonk et al. 2006). Htt has also shown involvement in Akt, activating pro-survival pathways, as well as inhibition of huntingtin-interacting protein 1 (HIPPI)

and pro-caspase 9, both involved in pro-apoptosis (Rigamonti, Bauer et al. 2000; Rigamonti, Sipione et al. 2001; Gervais, Singaraja et al. 2002; Humbert, Bryson et al. 2002; Rangone, Poizat et al. 2004).

1.2.2 Mutant Huntingtin Aggregation in Huntington's Disease

Normal and mutant htt are subject to proteolytic cleavage by caspases. Cleavage of htt liberates toxic fragments containing polyQ, and cellular toxicity increases in neuronal cells with accumulating htt fragments. Accumulation leads to activation of more protolytic caspases and the cell eventually dies (Goldberg, Nicholson et al. 1996). Studies of HD afflicted post mortem brain tissue showed mutant htt is more resilient to cleavage by caspase than normal htt, but other factors may be involved (Dyer and McMurray 2001). Caspase-6-mediated cleavage is thought to be a critical event in the disease process, and ongoing research is being conducted in YAC128 mouse models (Graham, Deng et al. 2006).

A key factor in the pathogenesis of HD is the accumulation of the polyQ extended huntingtin protein fragments as they build up in the cytoplasm and nucleus. Two major pathways explaining how polyQ expansion enables aggregation are in competition with each other. The first outlines aggregation of the polyQ stretch. Nuclear-growth polymerization involving a sustained lag-phase forms an initial aggregation nucleus. A fast extension phase occurs, and additional polyQ monomers join the emergent aggregate. The aggregate consists of beta-sheet rich fibrils and becomes a ribbon-like structure, which ultimately exhibits features of an amyloid (Wanker 2000; Bates 2003; Ross 2004).

The second pathway describes the formation of oligomers having the first 17 amino acid sequence and the polyQ sequence. As the polyQ sequence increases, the structure decompresses the oligomers then rearrange into an amyloid structure, resulting in aggregation (Thakur, Jayaraman et al. 2009).

1.2.3 Mutant Huntingtin and Excitotoxicity in Huntington's Disease

Striatal neurons become vulnerable to excitoxicity and dysfunction occurs at the corticostriatal synapses leading to neuronal death (Albin, Young et al. 1990). Abnormalities in glutamate transmission in post mortem human brain tissue have been studied, leading to the theory that excitoxicity is a major mechanism in the pathology of HD (DiFiglia 1990). Glutamate release from cortical afferents is critical for the survival and activity of striatal neurons; glutamate receptors are lost in HD and studies have found that NMDA receptor binding is significantly decreased (London, Yamamura et al. 1981; Young, Greenamyre et al. 1988; Albin, Young et al. 1990; DiFiglia 1990; Dure, Young et al. 1991). Altered glutamate release, and impairment of glutamate clearance from the synaptic cleft, may play a major role in excitotoxic neurodegeneration. Glial Glutamate transporter 1 (GLT1), one of the major glial glutamate transporters, may be altered, and consequently impairing regulation of synaptic transmission in HD (Tzingounis and Wadiche 2007; Sari, Prieto et al. 2010). Studies using HD mouse models R6/1 and R6/2 have demonstrated that GLT1 down-regulation may be responsible for the decrease in glutamate uptake by glial cells in the striatum (Lievens, Woodman et al. 2001; Behrens,

Franz et al. 2002; Shin, Fang et al. 2005; Miller, Dorner et al. 2008; Estrada-Sanchez, Montiel et al. 2009).

Glutamate may not be the only neurotransmitter associated in the pathogenesis of HD. Dopamine helps to directly regulate the release of glutamate from corticostriatal terminals by stimulation of dopamine, D2, receptors present on cortical afferents (Bamford, Robinson et al. 2004). In HD, downregulation of dopamine receptors D1 and D2 is found, which suggests the important role of the dopamine system in HD. Because of the ability of dopamine to directly regulate glutamate, reduction in the release of dopamine and reduction in the number of dopamine receptors affects the change in glutamate release. Degeneration of nigrostriatal projections in the substantia nigra, and overall decrease in dopaminergic neuron population is well established in HD (Ferrante, Kowall et al. 1987; Oyanagi, Takeda et al. 1989; Ginovart, Lundin et al. 1997; Suzuki, Desmond et al. 2001; Behrens, Franz et al. 2002; Yohrling, Jiang et al. 2003).

Excitotoxicity and apoptosis have also been linked to the dysfunction of intracellular calcium signaling, causing cytosolic and mitochondrial Ca^{2+} overload, in medium spiny neurons through increased sensitivity of NMDA receptors in the striatum (Bezprozvanny and Hayden 2004; Tang, Slow et al. 2005). Mutant htt binds to the inositol 1,4,5-triphosphate receptor 1 ($InsP_3R1$), resulting in increased Ca^{2+} release due to the stimulation of glutamate receptors known for activating the IP_3 signaling pathway (Tang, Tu et al. 2003; Tang, Slow et al. 2005). Mitochondrial Ca^{2+} storage eventually reaches maximum capacity, swells, and releases cytochrome c, Ca^{2+}, and other proapoptotic factors into the cytoplasm (Choo, Johnson et al. 2004; Zeron, Fernandes et al. 2004; Milakovic, Quintanilla et al. 2006). In addition, mutant htt acts directly on the

mitochondria decreasing the calcium threshold, further destabilizing the mitochondria (Zuccato, Valenza et al. 2010).

In addition to overloaded Ca^{2+} channels and mitochondrial dysfunction, oxidative stress also plays a large role in excitotoxicity (Dong, Wang et al. 2009). Oxidative stress has been implicated in neurodegenerative diseases, as it causes damage to motor neurons through the oxidation of lipids, proteins, and nucleic acids (Coyle and Puttfarcken 1993; Volterra, Trotti et al. 1994; Trotti, Danbolt et al. 1998). The body naturally defends against reactive oxygen species (ROS) responsible for oxidative stress through anti-oxidant mechanisms, such as the activation of nuclear factor erythroid 2-related factor 2 (Nrf-2) (Shinkai, Sumi et al. 2006). Nrf-2 induces and upregulates many Phase II enzymes, which scavenge for free radicals and interrupt the mechanism in which reactive chemicals work (Calkins, Johnson et al. 2009). Phase II enzyme inducers, or natural anti-oxidants, are being studied to evaluate their therapeutic potential (Zhang, Talalay et al. 1992; Zhao, Moore et al. 2005; Zhao, Kobori et al. 2006; Zhao, Moore et al. 2007; Danilov, Chandrasekaran et al. 2009; Tarozzi, Morroni et al. 2009). A naturally occurring compound found in broccoli and other cruciferous vegetables, Sulforaphane (Figure 1.1), has shown to be protective against oxidative stress by inducing Phase II enzymes, decreasing degradation of Nrf-2, and exert neuroprotection (Zhang, Talalay et al. 1992; Zhang, Lo et al. 2004; Shinkai, Sumi et al. 2006). Therefore, we chose to test Sulforaphane as a therapeutic, anti-oxidant to protect against neurodegeneration in HD.

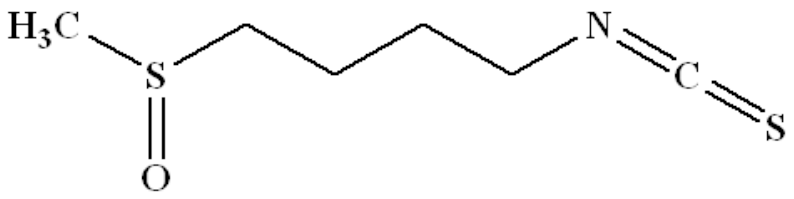

Fig 1.1 Sulphoraphane

1.3 Current Pharmacotherapy for Huntington's Disease:

To date, there is no cure for HD, or therapies that reverse symptoms. The search for pharmacological interventions is vast and ongoing, but there is no treatment to cease motor, cognitive, or psychological dysfunctions. The only FDA approved therapy specifically for those diagnosed with HD is Xenazine (tetrabenazine), for the treatment of chorea. Because of the vast neurodegeneration and resulting symptoms, the likelihood that one therapy will treat motor, psychological, and cognitive symptoms is exceedingly unlikely. At the present time, medical management of HD is attempted by treating each symptom area separately, although this is ineffective. For example, choreatic movements may be treated with tetrabenazine, psychological symptoms with neuroleptics, and cognitive symptoms with minocycline (Bonelli and Beal 2012). Another issue arises when looking at the side effects of the medications. Treatment of one symptom increases the prominence of another symptom. Chorea treatment therapies may worsen gait, increase parkinsonism, and increase apathy, as just one example (Burgunder, Guttman et al. 2011; Bonelli and Beal 2012).

Currently, treatments for HD related motor symptoms are minimal, and thus far there is little positive data for the treatment of chorea, the involuntary motor related movement of HD. Chorea may be partially managed with haloperidol, fluphenazine, or olanzapine, but overall, only symptomatic treatment is recommended when symptoms are disabling (Terrence 1976; Barr, Fischer et al. 1988; Gimenez-Roldan and Mateo 1989; Paleacu, Anca et al. 2002; Squitieri, Orobello et al. 2009). Neuroleptics, dopamine depleting agents, and atypical neuroleptics are most frequently prescribed for HD patients, and benzodiazepines are used as adjunctive therapy (Bonelli and Beal 2012). These may help control some motor symptoms and exacerbate others (Bonelli and Beal 2012). Typical neuroleptics work by blocking central dopamine receptors, but may worsen gait and swallowing, and cause tardive dyskinesia (Bonelli and Hofmann 2007). Thus far, medications resulting in action of GABAergic neurotransmission has failed to delay or attenuate the progression of HD. Benzodiazepines, such as clonazepam, are frequently used to treat chorea and anxiety (Frank and Jankovic 2010). Riluzole, a NMDA antagonist that interferes with glutamatergic neurotransmission, reducing excitotoxicity, increases serum concentrations of BDNF, and protects striatal neurons (Katoh-Semba, Asano et al. 2002; Bergerot, Shortland et al. 2004; Squitieri, Ciammola et al. 2008; Squitieri, Orobello et al. 2009). Riluzole is currently in phase II clinical trials for the treatment of HD related chorea.

The FDA approved drug tetrabenazine, binds to the central, presynaptic, intravesicular portion of the vesicular monoamine transporter, VMAT2, causing depletion of dopamine, norepinephrine, and serotonin (Asher and Aminoff 1981). Binding affinity is highest in the caudate nucleus, putamen, and the nucleus accumbens,

reaching the brain regions most impacted by HD (Mehvar and Jamali 1987; Thibaut, Faucheux et al. 1995). Unfortunately, tetrabenazine also causes parkinsonism, depression, anxiety, and insomnia, and therefore is not recommended in early/mild symptom stages (Jankovic and Beach 1997).

Many drugs have been used and explored for the treatment of the psychiatric implications related to HD, but there are no controlled clinical trials for depression or anxiety to date. Neuroleptics, selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants are typically prescribed to manage HD related symptoms; most popular medications being risperidone, and haloperidol [For review see ref. (Bonelli and Beal 2012)]. Information and studies regarding treatment of mania, apathy, anxiety, and obsessions related to HD is very scarce. When patients are unresponsive to pharmacological therapy, electroconvulsive therapy has been reported (Evans, Pedersen et al. 1987).

Current treatments for cognitive associated HD symptoms, mainly dementia, are mostly supportive, providing only mild cognitive benefits (Bonelli and Beal 2012). Riluzole, minocycline, and unsaturated fatty acids have been studied in open-label trials (Seppi, Mueller et al. 2001; Puri, Bydder et al. 2002; Vaddadi, Soosai et al. 2002; Bonelli, Heuberger et al. 2003). Cholinesterase inhibitors reached phase III clinical trials but proved to be ineffective in treatment of HD related cognitive decline (Fernandez, Friedman et al. 2000; de Tommaso, Specchio et al. 2004).

1.4 Role of Neurotrophic Factors in Huntington's Disease:

Neurotrophic factors, which are released from glial cells, neurons, and various other cells, promote growth and survival of developing neurons by preventing apoptosis (Deister and Schmidt 2006). Lack of neurotrophic factors results in neuronal death, which can lead to detrimental neurodegeneration affecting several target tissues (Appel 1981); thought to be a key factor in neurodegenerative diseases (Connor and Dragunow 1998; Murer, Yan et al. 2001; Kruttgen, Saxena et al. 2003). Neurotrophic factors can generally be categorized into three families: neurotrophins, glia cell-line derived neurotrophic factor family ligands (GFL) and neuropoietic cytokines (Deister and Schmidt 2006). Brain derived neurotrophic factor (BDNF), thought to be largely affected in HD, is part of the neurotrophin family, which are found in neurons and glial cells and medicated by tyrosine kinase (Trk) receptors [For review see ref. (Sari 2011). Other trophic factors, such as glial cell-line derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), and ciliary neurotrophic factor (CNTF), as well as short peptides with neuroprotective activity, for instance activity dependent neurotrophic factor (ADNF), also promote survival of neurons and regulate normal function in the central nervous system (CNS) (Anderson, Panayotatos et al. 1996; Brenneman and Gozes 1996; Alberch, Perez-Navarro et al. 2004).

1.4.1 Role of BDNF in Huntington's Disease

The ultimate function of a normal htt might be neuroprotection against cell death associated with a toxic stimulus, shown both in *vitro* and in *vivo;* htt may also increase transcription of BDNF, which is imperative to the survival of striatal neurons and integrity of corticostriatal synapses (Nakao, Brundin et al. 1995; Alcantara, Frisen et al. 1997; Hackam, Yassa et al. 2000; Rigamonti, Bauer et al. 2000; Leavitt, Guttman et al. 2001; Zuccato, Ciammola et al. 2001; Zhang, Li et al. 2003; Pascual and Guerri 2007; Paulsen, Langbehn et al. 2008; Aziz, Jurgens et al. 2009). BDNF plays an important role in supporting neuronal survival, enhancing synaptic plasticity, and inducing neuronal outgrowth; we can see how disturbances can have a major neurological impact in HD. BDNF is found to be co-localized with htt in cortical neurons that extend to the striatum (Pillai, Hansen et al. 2012). Cortical BDNF is anterogradely transported into vesicles along the corticostriatal afferents, released into axon terminals, captured by striatal neurons, resulting in rapid intracellular signals (de la Monte, Vonsattel et al. 1988; Halliday, McRitchie et al. 1998; Paulsen, Langbehn et al. 2008). BDNF also controls glutamate release at the corticostriatal synapse (Aziz, Jurgens et al. 2009). Normal htt is thought to regulate the activity of the BDNF promoter II, affecting BDNF transcription (Zuccato, Ciammola et al. 2001; Zuccato, Tartari et al. 2003). When normal htt is reduced in HD, BDNF cannot support its striatal targets due to the decreased stability of the cortical afferents. Accumulation of mutant htt can lead to decreased BDNF in the striatum, and reduced BDNF transcription; the mutation will also reduce BDNF vesicle transport along microtubules (Volterra, Trotti et al. 1994).

Increased accumulation of repressor REST/NRSF that normally regulates gene expression during transition from immature to mature neurons (Ooi and Wood 2007), in the nucleus binds to and activates the RE1/NRSE silencer causing a reduction in transcription of BDNF (Zuccato, Belyaev et al. 2007; Zuccato and Cattaneo 2007). BDNF transcription may also be affected in HD by modified transcriptional events of cAMP response element (CREB) and CREB binding protein (CBP) that regulate BDNF promoter activity (Cha 2007). The reduction in transcription may be due to a deficit in accessibility that arise when mutant htt interacts with glutamine-rich activation domains and the acetyltransferase domain of CBP, resulting in a more compact chromatin structure caused by a reduction in histone acetylation; therefore leading to reduced BDNF mRNA levels (Steffan, Bodai et al. 2001).

Decreased regulation of BDNF vesicle transport along microtubules may be a result of a reduction in phosphorylation of htt at serine-421 by Akt kinase (Colin, Zala et al. 2008). Phosphorylation at Serine-421 produces anterograde transport of BDNF by htt. When htt is not phosphorylated in the striatum due to the polyQ expansion, retrograde transport of BDNF vesicles may occur; and this reduction in phosphorylated htt has been found in animal models of HD and post mortem human tissues (Warby, Chan et al. 2005; Colin, Zala et al. 2008). Reduction of BDNF increased vulnerability of striatal neurons, including striatal medium spiny neurons, and dysfunction of dopaminergic neurons, contributing to development of HD [For review see ref.(Cattaneo, Zuccato et al. 2005)].

1.4.2 Role of GDNF in Huntington's Disease

GDNF, similar to neurotrophins, is mediated by Trk receptors and provides neuroprotection to striatal, medium spiny neurons (Glass and Yancopoulos 1993; Perez-Navarro, Arenas et al. 1996; Chiba, Nishimoto et al. 2007). GDNF indirectly protects neurons by binding to the GFRα1/Ret receptor complex, activating PI3K/Akt pathway, inhibiting caspases 9 and 3, and subsequently inhibiting cell death (Hamanoue, Takemoto et al. 1996; Humbert, Bryson et al. 2002). GDNF is considered to provide neuroprotection prior to the formation of inclusions of mutant htt in the striatum, but studies in mice have shown that administering GDNF after inclusion formation has occurred renders inadequate (Popovic, Maingay et al. 2005; Sari 2011).

1.4.3 Role of FGF in Huntington's Disease

FGF-2 exhibits neuroprotection via use of high affinity Trk receptors, protecting neurons from toxins and excitatory amino acids (Haque and Isacson 2000). FGF-2 has been found to upregulate normal htt in a dose-dependent approach, increasing neurogenesis and stimulating neuroprotection (Haque and Isacson 2000; Jin, LaFevre-Bernt et al. 2005). These studies in HD mouse models have proved encouraging in FGF-2s ability to replace lost neurons in HD.

1.4.4 Role of CNTF in Huntington's Disease

Providing neuroprotection for striatal neurons though stimulation of gene expression, cell survival, and cell differentiation, CNTF is part of the IL-6 cytokine superfamily (Linker, Maurer et al. 2002; Stankoff, Aigrot et al. 2002). CNTF has shown promising results in preclinical and clinical studies preventing neurodegeneration of neurons in the striatum and cortex for the treatment of HD patients (Emerich, Winn et al. 1997; Bloch, Bachoud-Levi et al. 2004).

1.4.5 The Potential Role of Colivelin in Huntington's Disease

Colivelin is a hybrid peptide developed to take advantage of the active properties of humanin (HN) and activity dependent neurotrophic factor 9 (ADNF-9) (Chiba, Yamada et al. 2005). ADNF-9 is fused at the C-terminus to potent HN derivative AGA-(C8R) HNG17 to create the novel hybrid peptide, Colivelin. HN is an anti-apoptotic, rescue factor that has shown to be protective against the toxic insults that occur in human neurodegenerative disease (Sponne, Fifre et al. 2004). ADNF-9, derived from ADNF, also known as SAL (SALLRSIPA), is the active core of its parent compound and mimics the neuroprotective activity (Brenneman and Gozes 1996; Brenneman, Hauser et al. 1998). ADNF-9 has been found to protect against Aβ in Alzheimer's disease (AD), apolipoprotein E deficiencies, and oxidative insults, as well as enhancing synapse formation (Brenneman and Gozes 1996; Bassan, Zamostiano et al. 1999; Glazner, Boland et al. 1999; Blondel, Collin et al. 2000).ADNF-9 is active in the femtomolar concentration range, but activity is lost by about 10 nM, whereas humanin analogue,

AGA(C8R)HNG17, is active starting at 10 pM (Brenneman and Gozes 1996; Brenneman, Hauser et al. 1998; Chiba, Hashimoto et al. 2004; Chiba, Yamada et al. 2005). Increased stability and ability to cross the blood brain barrier are two additional advantages to the combination of ADNF-9 and HN (Matsuoka, Hashimoto et al. 2006).

Colivelin (Figure 1.2) is active in the femtomolar range and does not lose activity at higher concentrations (Chiba, Yamada et al. 2005). Humanin was found to provide neuroprotection against AD related insults, such as Aβ neurotoxicity (Hashimoto, Niikura et al. 2001). The JAK2/STAT3 signaling pathway showed importance in colivelin neuroprotection against AD-related memory loss (Yamada, Chiba et al. 2008; Chiba, Yamada et al. 2009; Chiba, Yamada et al. 2009). In vivo study found that colivelin has more potent neuroprotective effect than humanin and ADNF-9, when tested against Aβ neurotoxicity (Chiba et. al. 2005). Colivelin neuroprotection was associated with motor performance improvement but not lifespan in Amyotrophic lateral sclerosis (ALS) mouse model of SOD1 (Chiba et. al. 2004). Moreover, another study showed that colivelin improved motor performance and lifespan against FSOD1 as well as suppressing motoneuronal death (Chiba, Yamada et al. 2006). Through this previous research, it seemed reasonable to study the effects of colivelin in other neurodegenerative diseases, such as HD.

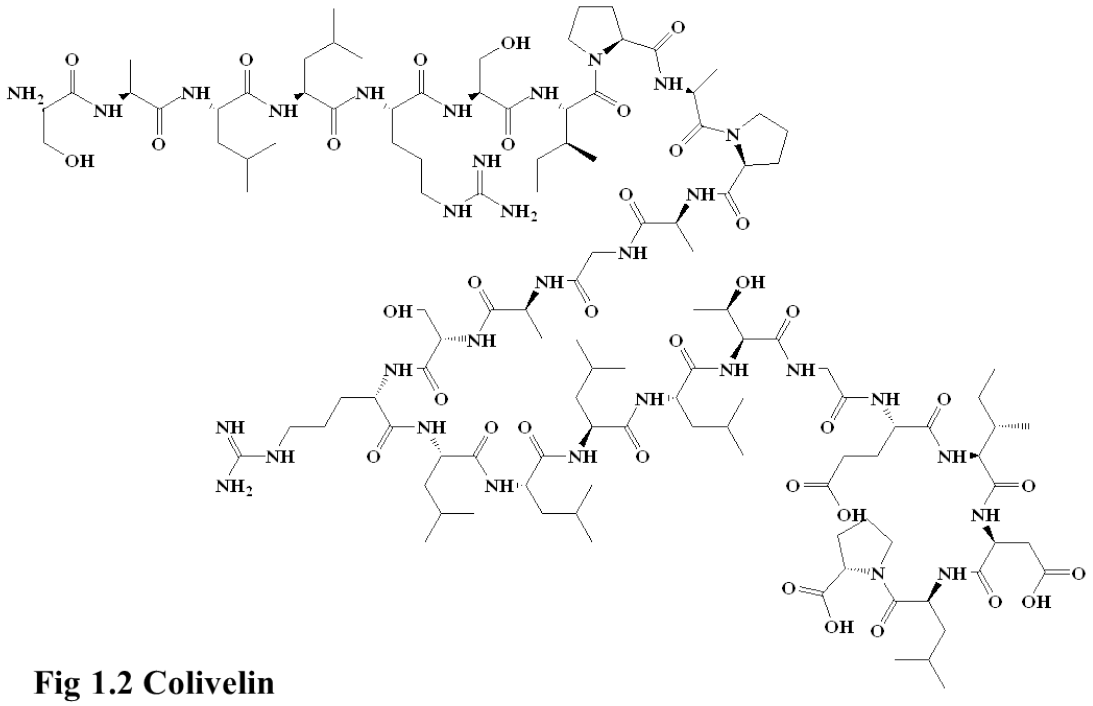

Fig 1.2 Colivelin

1.5 R6/2 Mouse Model of Huntington's Disease:

Several mouse animal models for the study of HD are available including R6/1, R6/2, YAC72, YAC128, Tg100, BACHHD, CAG71, and CAG94; representing transgenic, knock-in, and conditional mouse models (Mangiarini, Sathasivam et al. 1996; Hodgson, Agopyan et al. 1999; Laforet, Sapp et al. 2001). These animals allow extensive study of the pathogenesis of HD, as they chronically progress though the disease and have the ability to display cause-and–effect relationships. (Brouillet, Conde et al. 1999; Menalled and Chesselet 2002; Hickey and Chesselet 2003; Levine, Cepeda et al. 2004). The different models provide different expression of HD. The transgenic mice that express N-terminal fragments of exon 1, and a pathologically relevant length of polyQ repeats, display the phenotype of a rapidly progressing form of the disease. They display

the motor degeneration and weight loss associated with the disease. Transgenic mice carrying the full length mutant htt, as seen in humans, exhibit a more prolonged form of HD; the motor degeneration is reduced but the neuronal degeneration is more characteristic of human HD [For review see ref. (Kumar, Kalonia et al. 2010)]. In our study, we chose transgenic R6/2 mice due to its rapid degeneration and expressive motor deficits.

The R6/2 mice begin to display symptoms associated with neurodegeneration by age week six, specifically involuntary and choreatic movements, tremors, seizures, weight loss, and abrupt sudden death due to unknown cause (Hurlbert, Zhou et al. 1999). These mice carry between 115- 125 CAG repeats (Mangiarini, Sathasivam et al. 1996). Much like human HD patients, R6/2 mice also develop ubiquitinated inclusions, and other early brain changes, that appear before motor and cognitive deficits are noticeable (Bibb, Yan et al. 2000; Morton, Lagan et al. 2000; Gibson, Reim et al. 2005). Aggregate formation in the different regions of the brains appears to be precisely timed in this mouse model, developing in the cortex prior to formation in the striatum; mutant htt aggregation can be found by age day 14 preceding inclusion ubiquitination. It is important to note that all HD transgenic mouse models develop ubiquitinated inclusions (Gong, Kielar et al. 2012).

1.6 Aims and Objectives:

The aim of this study was to investigate the effects of trophic peptides for the attenuation of HD symptoms. The aim was to determine the preventative effects if target drugs were administered early in disease progression using mouse model R6/2. By measuring motor function and animal weight we were able to evaluate the behavioral abnormalities found in HD. We proposed the use of two doses of the anti-oxidant compound Sulforaphane (SULF), administered over a period of 14 days to reduce oxidative stress, thereby reducing cell death in the brain. We proposed the evaluation of ceftriaxone (CEF) (Figure 1.3), known to upregulate and activate GLT1 in the CNS. Finally, we proposed the administration of Colivelin (Coliv), and later its analogue dPEG Colivelin (dPEG), to treat HD symptoms through prevention of cell death. Through the comparison of saline treated animals and comparison to wild-type R6/2 mice, we hypothesize that the administration of these drugs may delay the onset and/or decrease the severity of HD symptoms, as well as reduce characteristic body weight loss.

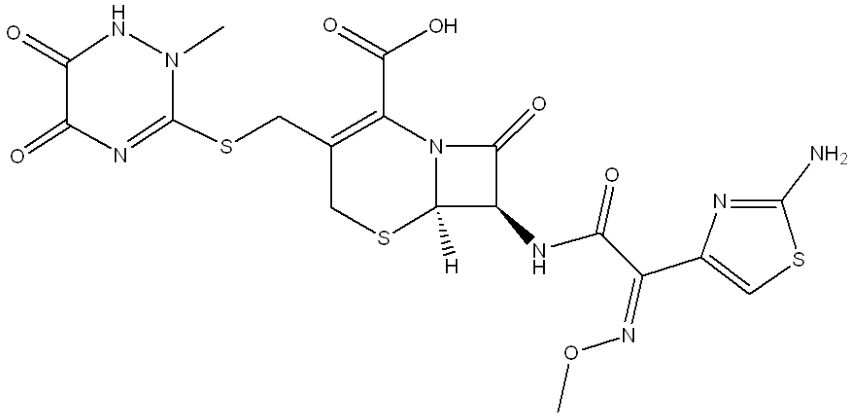

Fig 1.3 Ceftriaxone

Chapter 2

Methods and Materials

2.1 Subjects

Male R6/2 mice were used as they are an established model of HD. They were purchased from The Jackson Laboratory (Bar Harbor, ME) at 28-35 days of age. They were single housed in standard plastic tubs in the DLAR (Department of Laboratory Animal Resources, University of Toledo, HSC) and acclimatized to the vivarium. The plastic tubs had Corn-cob bedding and a temperature of 21°C maintained in the room. A 12 hour light/dark cycle was followed. Food and water were given *ad libitum*. After habituation to the vivarium, the animals were given a baseline motor test on a rotarod (Columbus Instruments) and weighed. They were injected i.p. with the assigned treatment beginning within their sixth week of age.

2.2 Behavior Testing

Motor performance and weight were evaluated twice per week for two weeks. Four measurements were performed per treatment group. Male R6/2 mice were exposed

to the rotarod for one baseline trial before administration of therapy. Rotarod trial one (R1) was evaluated once for each treatment group between the first and fourth day of treatment. Rotarod trial two (R2) was evaluated once for each treatment group between the fourth and ninth day of treatment. Rotarod trial three (R3) was evaluated once for each treatment group between the seventh and eleventh day of treatment. Rotarod trial four (R4) was evaluated once for each treatment group on treatment day fourteen. Each animal was subjected to three consecutive rotarod trials and the latency values were recorded. The motor performance was evaluated by the mean of the three trials.

The mice were tested in sets of four animals; each mouse was placed on a balance to record the body weight, and then placed in the assigned chamber of the rotarod. Once all animals were placed on their prospective rods, the apparatus was turned on. There was a ten second latency prior to rotation. Every ten seconds, the rod increased by one rotation per minute (rpm) to a maximum speed of 60 rpm. When all four mice had fallen from the rotating rods, the latency times were recorded, and the machine was reset for the next trial. A one minute interval was given between trials during which time the mouse stayed in their assigned chamber. After the last trial, each mouse was placed back in their appropriate cage, and the rotarod was cleaned to eliminate odors between sets of animals.

The clasping score of each animal was also evaluated. Clasping indicates neurological impairments and the phenotype presents when the mouse is suspended by the tail, pressing its paws against the body. Each mouse was suspended by the tail for 30 seconds to elicit the phenotype, where the animal. We scored the animal on a scale of zero to four, where zero was no clasping paws, one was one clasping limb, two was two clasping limbs, three was three clasping limbs, and four was all limbs clasping.

Lastly, we performed another motor examination named the vertical pole descent, based on the same test performed in the R6/2 mouse model by Simmons, et. al. (Simmons, Mehta et al. 2011). We built an apparatus consisting of a coarse wooden pole, height: 55cm diameter: 1cm, anchored by a circular wooden base, height: 2.5 cm diameter: 45 cm, according to the specifications outlined by Simmons. The mouse was placed at the top of the pole, the recorded time began when the mouse's tail was released and ended when the mouse placed both hind feet, all four paws, on the floor of the apparatus. If the mouse could not complete the task, or fell off of the pole, a default time of 60s was recorded. The mice were subjected to four trials and the fastest of the four attempts were taken to be analyzed.

2.3 Treatment groups

Starting week six, the drugs were administered intra-peritoneally (i.p.) to the animals for 14 days after assigning them to different treatment groups randomly. The groups included Saline (n=10 wild type; n=11 transgenic R6/2), Sulforaphane 5 mg/kg (n=4 wild type; n=4 transgenic R6/2), Sulforaphane 10 mg/kg (n=8 wild type; n=9 transgenic R6/2), Colivelin 40 mg/20g (n=8 wild type; n=8 transgenic R6/2), dPEG12-Colivelin 40 mg/20g (n=4 wild type; n=11 transgenic R6/2), and Ceftriaxone 100 mg/kg (n=15 wild type; n=15 transgenic R6/2). The reconstitutions of the compounds are described as follows:

1) Sulforaphane was purchased from LKT Laboratories, Inc. as a powder in 25 mg bottles. It was reconstituted with saline and calculations were made according to the animal weight to administer the doses of 5 mg/kg and 10 mg/kg. 2) Colivelin and dPEG-Colivelin were purchased through Ohio Peptide, LLC and reconstituted with saline, with the exception of subjects 201-208 who were administered doses reconstituted with phosphate buffered saline solution. The dilution was made in such a way as to administer a 40 mg/20g dose, adjusted according to the animal weight. Colivelin and dPEG-Colivelin were purchased as a powder in 10 mg bottles. 3) Ceftriaxone was purchased as powder in 250 mg bottles from the pharmacy at UTMC. It was reconstituted with saline and calculations were made according to the animal weight to administer the doses of 100 mg/kg.

2.3.2 Brain tissue harvesting:

After completion of study, all animals were euthanized by exposure to CO_2, followed by cervical dislocation. The animals were then decapitated, and the brains were dissected out, frozen immediately on dry ice and stored at -70°C. The brains were then further dissected into left and right parts of two areas, prefrontal cortex (PFC), and striatum (ST) based on the coordinates using a cryostat (Leica) and stored for immunoblotting. We also procured a tail tissue sample for genotyping.

2.4 Immunoblotting:

2.4.1 Grouping

Western blot assays were performed on the PFC, and the ST to determine the *p*-AKT and *p*-JNK protein levels. The comparisons were made between the saline, dPEG Colivelin 40mg/20g, and Colivelin 40mg/20g. The tissue samples were homogenized immediately using the RIPA lysis buffer.

2.4.2 Protein Homogenization

Lysis buffer was prepared by mixing 1mM PMSF, 2mM sodium orthovanadate, 20mM sodium pyrophosphate and protease inhibitor tablet dissolved in RIPA Cell Lysis Buffer 2. The amount of lysis buffer used was adjusted based on the size of the sample and it was ground with a pestle until no solid mass remained. The homogenized suspension was incubated on ice for 30 minutes and then centrifuged at 13,200 rpm for 15 minutes in a 4°C refrigerated micro-centrifuge. For each sample, the supernatant was aliquoted into four labeled polypropylene tubes and the pellet was discarded. One aliquot of each sample was used to perform the protein quantification assay.

2.4.3 Protein Quantification Assay

To determine the amount of protein present in the homogenized samples the Lowry Protein Quantification assay was performed using 96 well plates. The protein

quantification values were used to calculate the appropriate dilutions such that all the samples used for Western blot assays had the same concentration of protein per unit volume. Bovine serum albumin (BSA) (1.48 mg/ml, New England Biolabs) was used as the standard and serial dilutions of BSA were made to produce a linear standard curve. The lysis buffer was used to make up the volume and all standards and samples were assayed in triplicates. Biorad Protein Assay Reagent A and Reagent S (Biorad laboratories) were combined in the appropriate concentrations, added to all the wells and mixed thoroughly. Reagent B was added as required, the plate was incubated at room temperature for 15 minutes before reading the optical density. The optical density was read at 750 nm wavelength and normal speed using the Multiskan FC spectrophotometer (Thermo Scientific). A standard curve was calculated by plotting the BSA optical density values against the known concentrations; the regression and line equation was obtained. The concentration of the protein in the samples was then calculated using their optical density and the equation of the line.

2.4.4 Western Blot Procedure

2.4.4.1 Preparation of samples

Samples were divided into three groups: saline, dPEG-Colivelin, and Colivelin. The samples were thawed on ice and diluted with lysis buffer based on their protein quantification values to achieve a concentration of 12 µg/20 µl. A 5X Laemelli dye (1M Tris HCl, 100% Glycerol, SDS, bromophenol blue, b-mercaptoethanol) was added to the sample (12 µl/ 20µl of the diluted sample), mixed via pipette and thoroughly vortexed.

The sample mixture was then incubated at 98°C for four minutes in a digital dry bath (Labnet International Inc.). The vials were opened and resealed to relieve pressure after the first minute, and vortexing was repeated at the end of the total four minutes. The sample mixture was centrifuged at 4°C and 13,200 rpm for 4 minutes (Centrifuge 5415R, Eppendorf Inc.).

2.4.4.2 Electrophoresis:

Tris-glycine gels (10-20%; 1.0mmX12 well, Invitrogen) were placed in an electrophoresis apparatus and submersed in 1X Laemmli buffer (Tris base, glycine, SDS and deionised water). The wells of the gel were flushed to avoid air bubbles. The centrifuged sample mixture was used for the procedure. A total of 20 µl of the mixed samples was added to each well and electrophoresis was executed at 200 volts for one hour.

2.4.4.3 Transfer of proteins:

The separated proteins were transferred from the gels onto immobilon-P membranes in a transfer apparatus. The membranes were placed on the gels and sandwiched between an anode and a cathode plate that were further separated by layers of Whatman paper and sponges soaked in transfer buffer. The transfer box was also filled with Transfer buffer (deionised water, glycine, tris base, and methanol) so that the whole

assembly was submerged. The set-up was run at 24 volts for 2.5 hours. Upon completion of the run, the membranes were numbered, and placed in petri dishes.

2.4.4.4 Primary antibody

The membranes were washed immediately with deionised water until the dye disappeared and blocked with blocking buffer (dry milk and 1X TBST) and placed on agitator. After 30 minutes, the primary antibody *p*-Akt (AB 4060 Phospho-Akt (Ser473) XP Rabbit, Cell Signaling Tech., Inc.) was added at 1:2000 dilutions. The petri dishes were sealed with parafilm and incubated overnight in the refrigerator, to ensure stability of the antibody and prevent contamination, by shaking at 150 rpm.

2.4.4.5 Secondary antibody

The blocking buffer from the overnight incubation was poured out and the membranes were washed with 1X TBST (Tris base, sodium chloride, and deionised water at pH adjusted to 7.4) five times for five minutes each. The membranes were then blocked again with blocking buffer for 30 minutes and incubated with the appropriate dilution of secondary antibody for 1.5 hours (for *p*-Akt and *p*-JNK, anti-rabbit IgG [Thermo Scientific 31458]. The membranes were washed again with 1X TBST, five times for five minutes each, and prepared for developing.

2.4.4.6 Developing

The washed membranes were dried and used for chemiluminiscent detection. They were incubated with developer solution, a 1:1 ratio mixture of SuperSignal West Pico Luminal enhancer and SuperSignal West Pico stable peroxidase solution (Developer kit, Pierce) for approximately one minute. The membranes were dried a second time, placed between transparent sheets and exposed to HyBlot CL Film (Denville Scientific) in a cassette for two to twenty minutes depending on the signal strength. The exposed films were developed with an SRX-101A machine.

2.4.5 Normalization of Protein Loading

The procedure of incubating with antibodies and developing was repeated using β-tubulin primary antibody and anti-mouse secondary antibody in 1:5000 dilution for all blots. Each membrane was also washed five times with 1X TBST, incubated with antibodies and developing was repeated for the corresponding secondary antibody, total-Akt or total-JNK, and anti-mouse secondary antibody in 1:5000 dilutions.

2.4.6 Analysis of the Western Blots

In order to quantify the digitized images, the developed films were analyzed with the help of SRX-101A Film processor and MCID software. The size and density of the bands obtained for *p*-Akt, total-Akt, *p*-JNK, total-JNK, and β-tubulin were measured for

all study groups. Photomicrographs for *p*-Akt, Akt and β-tubulin bands were taken using the Q imaging DC camera and QCID software.

2.5 Statistical Analysis

The rotarod motor test results were normalized to a percent baseline for each rotarod trial (B, R1-R4). Independent t-tests were used to detect differences between treatments at specific rotarod trials. Repeated measures ANOVA were used to compare different drug treatments over all rotarod trials. Statistics for rotarod motor tests were conducted using SPSS version 20. The ratios of p-Akt/Akt, or p-JNK/JNK were calculated and one-way ANOVA was employed to determine differences between the control and treatment groups using GraphPad Prism version 5 statistical software. Statistical significance was set at $p< 0.05$ for all analyses.

Chapter 3

Results

3.1 Colivelin Groups

3.1.1 Effect of Colivelin treatment on motor performance

Rotarod motor performance measurements taken before and after treatment were compared to determine if Colivelin could significantly improve the motor deficits as a result of HD. Rotarod trial times were taken in seconds for each mouse, then normalized to a percent baseline number in order to control for variability that occurs with different animal subjects. Baseline (B) motor tests were taken one day prior to beginning treatment. All subsequent rotarod trials are denoted using R1, 2, 3, and 4, as they represent a range of days. Figure 3.1 shows the average percent baseline of rotarod/motor performance within each group plotted and four different rotarod trials. The treatment groups were: Saline (n=10), and Colivelin (n=8).

Independent T-test analysis, comparing saline (M= 1.298, SEM= 0.107) to colivelin treatment (M= 0.70, SEM= 0.8526), Colivelin treatment decreased rotarod performance $t(8) = 4.366$, $p<0.05$.

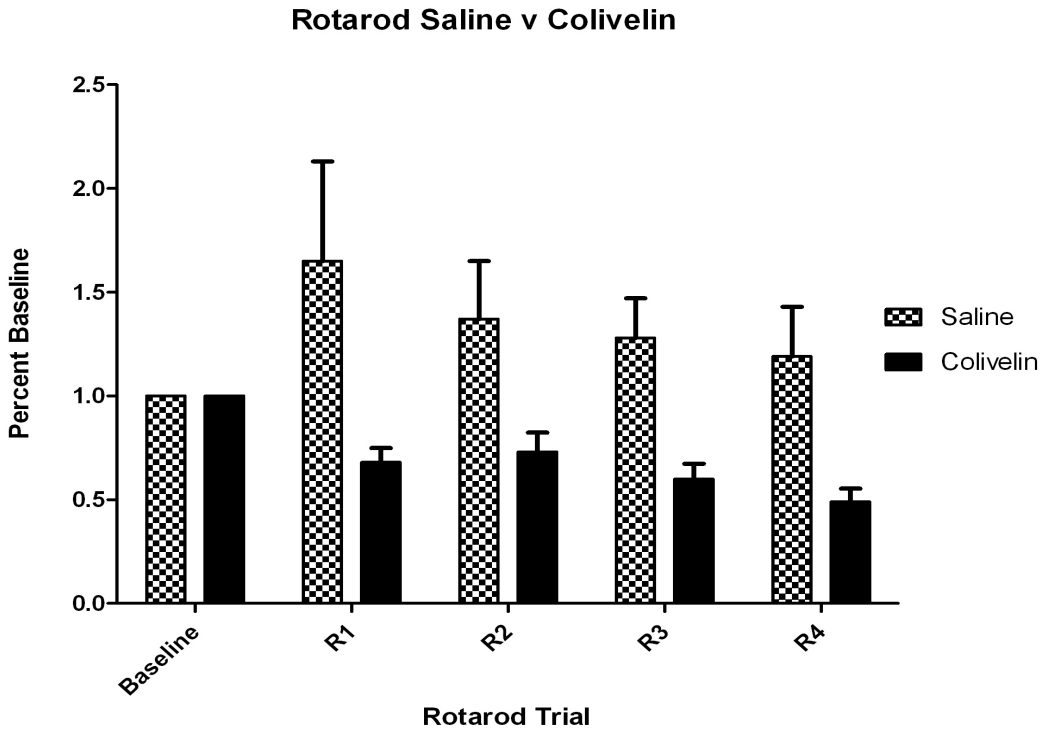

Figure 3.1 Bi-weekly rotarod motor performance of male R6/2 mice treated for 14 days with 40 mg/20g (i.p.) Colivelin (n=8), or saline (n=10). Graph represents average rotarod performance expressed in percent baseline (±SEM) one day prior to treatment and during the treatment (days 1-14). Independent t-test analysis revealed Colivelin treatment decreased rotarod performance $t(8) = 4.366$, $p<0.05$.

3.1.2 Effect of dPEG-Colivelin treatment on motor performance

Rotarod motor performance measurements were taken before and after treatment and compared to determine if dPEG-Colivelin could significantly improve HD related motor dysfunction. dPEGylation was thought to improve upon the effects of Colivelin administration. Rotarod trial times were taken in seconds for each mouse, and then normalized to a percent baseline number. Baseline (B) motor tests were taken one day prior to beginning treatment. All subsequent rotarod trials are denoted using R1, 2, 3, and 4, as they represent a range of days. Figure 3.2 shows the average percent baseline of

rotarod/motor performance within each group plotted and four different rotarod trials. The treatment groups were: Saline (n=10), and dPEG-Colivelin (n=11).

Independent T-test analysis, comparing saline to dPEG-Colivelin treatment, revealed no improvement in dPEG-Colivelin treated animals.

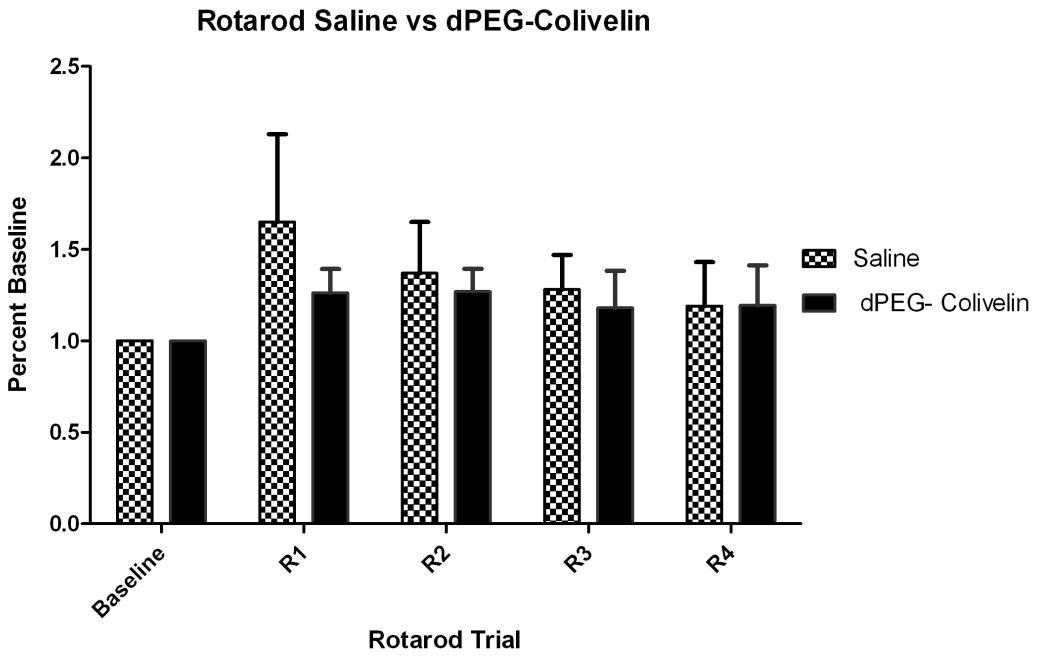

Figure 3.2 Bi-weekly rotarod motor performance of male R6/2 mice treated for 14 days with 40 mg/20g (i.p.) dPEG-Colivelin (n=11), or saline (n=10). Graph represents average rotarod performance expressed in percent baseline (±SEM) one day prior to treatment and during the treatment (days 1-14). Independent t-test analysis revealed no significance difference among control and treatments group.

3.1.3 Comparison of Colivelin and dPEG Colivelin treatment on motor performance

Repeated measures ANOVA were performed to detect main (time) and interaction (treatment X time) effects when comparing rotarod trials within the Colivelin, dPEG-Colivelin, and saline treated groups. This analysis revealed no effect on the motor performance capabilities of Colivelin or dPEG-Colivelin treated R6/2 mice.

3.1.4 Effect of Colivelin or dPEG-Colivelin treatment on Akt expression

We further determined whether Colivelin treatment involves some of the neuroprotective signaling pathways in the HD R6/2 mouse model. The expression of Akt kinase was compared in saline, dPEG-Colivelin, and Colivelin treated R6/2 mice to determine the signaling pathway involved in these treatments. Western blot was used to determine the effects of dPEG-Colivelin and Colivelin on Akt kinase in the PFC and ST. Figure 3.3A shows immunoblots for *p*-Akt, total-Akt, and β-tubulin in saline, dPEG-Colivelin, and Colivelin. One-way ANOVA analysis, followed by a posthoc Dunnett multiple comparisons test, did not reveal any significant difference between all groups in the PFC (Figure 3.3B).

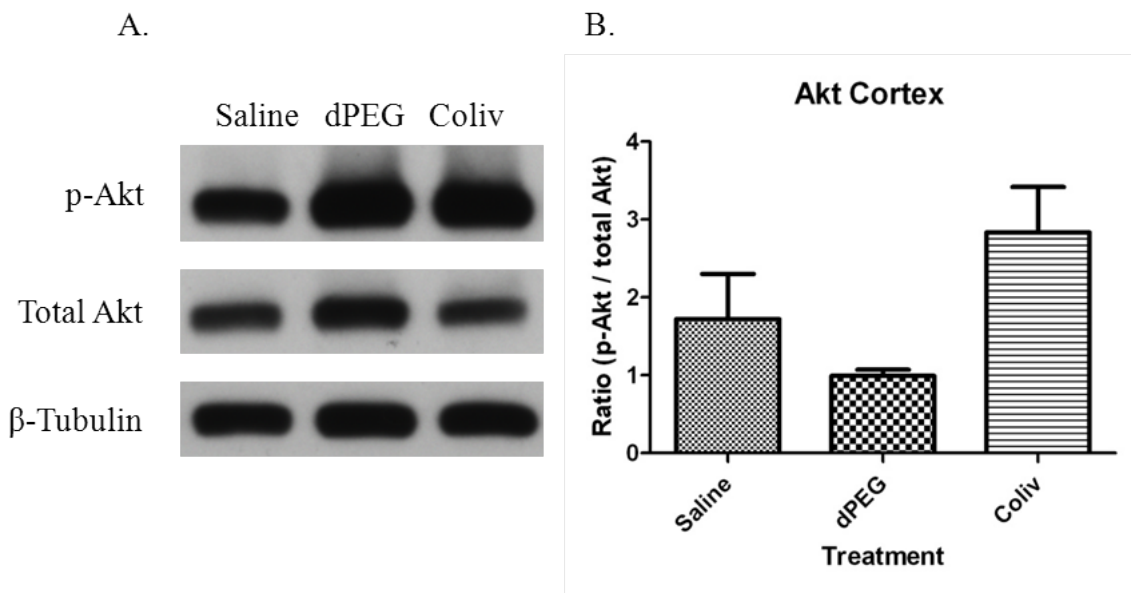

Figure 3.3 Effects of 40 mg/20g (i.p.) Colivelin (n=4), 40 mg/20g (i.p.) dPEG-Colivelin (n=4), and saline (n=4) groups on Akt expression in PFC. A) Each panel presents immunoblots for *p*-Akt, total Akt, and β-tubulin, which was used as a control loading protein. B) Quantitative analysis revealed no significant difference in the ratio of p-Akt/Total Akt in Colivelin group, or dPEG-Colivelin group, as compared to the saline vehicle group. Error bars indicate SEM.

Figure 3.4A display immunoblots for *p*-Akt, total-Akt, and β-tubulin for all groups in the ST. Figure 3.4B depicts the results of the one-way ANOVA, followed by a posthoc Dunnett multiple comparisons test, which shows a significant difference, $F(2,9) = 15.88$, $p<0.05$, in the expression of Akt in the ST between saline, dPEG-Colivelin, and Colivelin treatments. This result indicates that Colivelin treatment of R6/2 mice upregulates the expression of Akt in the ST as compared to saline and dPEG-Colivelin treated animals.

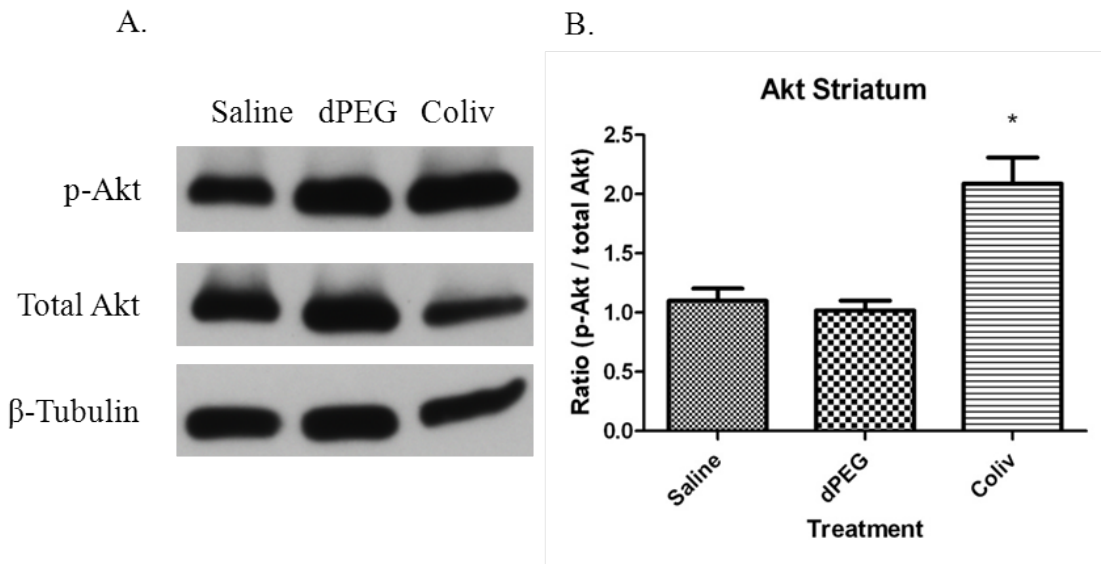

Figure 3.4 Effects of 40 mg/20g (i.p.) Colivelin (n=4), 40 mg/20g (i.p.) dPEG-Colivelin (n=4), and saline (n=4) groups on Akt expression in ST. A) Each panel presents immunoblots for *p*-Akt, total Akt, and β-tubulin, which was used as a control loading protein. B) Quantitative analysis revealed a significant increase in the ratio of *p*-Akt/Total Akt in Colivelin group as compared to the saline and dPEG-Colivelin treatment groups. Error bars indicate SEM. (* $p<0.05$).

3.1.4 Effect of Colivelin treatments on JNK expression

The expression of JNK was compared in saline, dPEG-Colivelin, and Colivelin treated R6/2 mice to determine if treatment could reduce the effects of neuronal death. Figure 3.5A shows immunoblots for *p*-JNK, total-JNK, and β-tubulin in the PFC. Figure 3.5B depicts the results of the one-way ANOVA, followed by a posthoc Dunnett multiple comparisons test, which shows a significant difference, $F(2,9) = 8.636$, $p<0.05$. This result indicates that Colivelin treatment of R6/2 mice upregulates the expression of JNK in the PFC as compared to saline and dPEG-Colivelin treated animals.

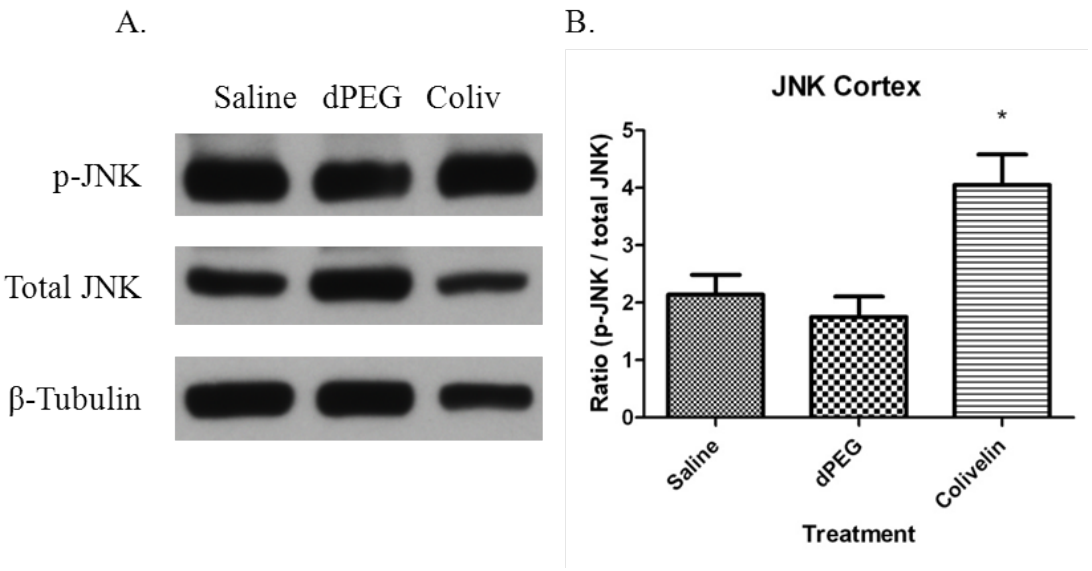

Figure 3.5 Effects of 40 mg/20g (i.p.) Colivelin (n=4), 40 mg/20g (i.p.) dPEG-Colivelin (n=4), and saline (n=4) groups on JNK expression in PFC. A) Each panel presents immunoblots for *p*-JNK, total JNK, and β-tubulin, which was used as a control loading protein. B) Quantitative analysis revealed a significant increase in the ratio of *p*-JNK/Total JNK in Colivelin group as compared to the saline and dPEG-Colivelin treatment groups. Error bars indicate SEM. (* $p<0.05$).

Figure 3.6A displays the immunoblots for *p*-JNK, total-JNK, and β-tubulin in the ST. Figure 3.6B depicts the results of the one-way ANOVA, followed by a posthoc Dunnett multiple comparisons test, which shows a significant difference, $F(2,9) = 9.264$, $p<0.05$. These results indicate that Colivelin treatment of R6/2 mice upregulates the expression of JNK in the PFC and ST as compared to saline treated animals.

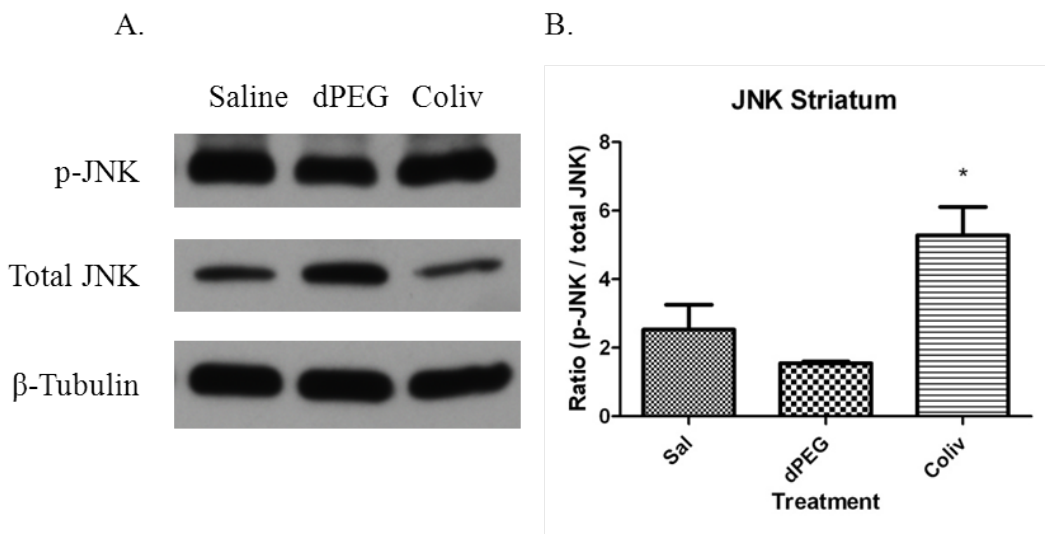

Figure 3.6 Effects of 40 mg/20g (i.p.) Colivelin (n=4), 40 mg/20g (i.p.) dPEG-Colivelin (n=4), and saline (n=4) groups on JNK expression in ST. A) Each panel presents immunoblots for *p*-JNK, total JNK, and β-tubulin, which was used as a control loading protein. B) Quantitative analysis revealed a significant increase in the ratio of *p*-JNK/Total JNK in Colivelin group as compared to the saline vehicle group. Error bars indicate SEM. (* $p<0.05$).

3.2 Ceftriaxone Groups

3.2.1 Effect of Ceftriaxone treatment on motor performance

Rotarod motor performance test was measured before and during treatment with ceftriaxone to determine if ceftriaxone attenuated motor deficiencies caused by HD. The rotarod trials were measured in seconds and normalized to a percent baseline number to account for variability from subject to subject. Baseline (B) motor tests were taken one day prior to beginning treatment. All subsequent rotarod trials are denoted using R1, 2, 3, and 4, as they represent a range of days. Figure 3.7 shows the average rotarod performance by group, Ceftriaxone treated compared to saline treated, plotted against

percent baseline. The treatment groups were: saline (n=10), and Ceftriaxone (n=15). T-test analyses were performed, finding no significant difference between the two groups.

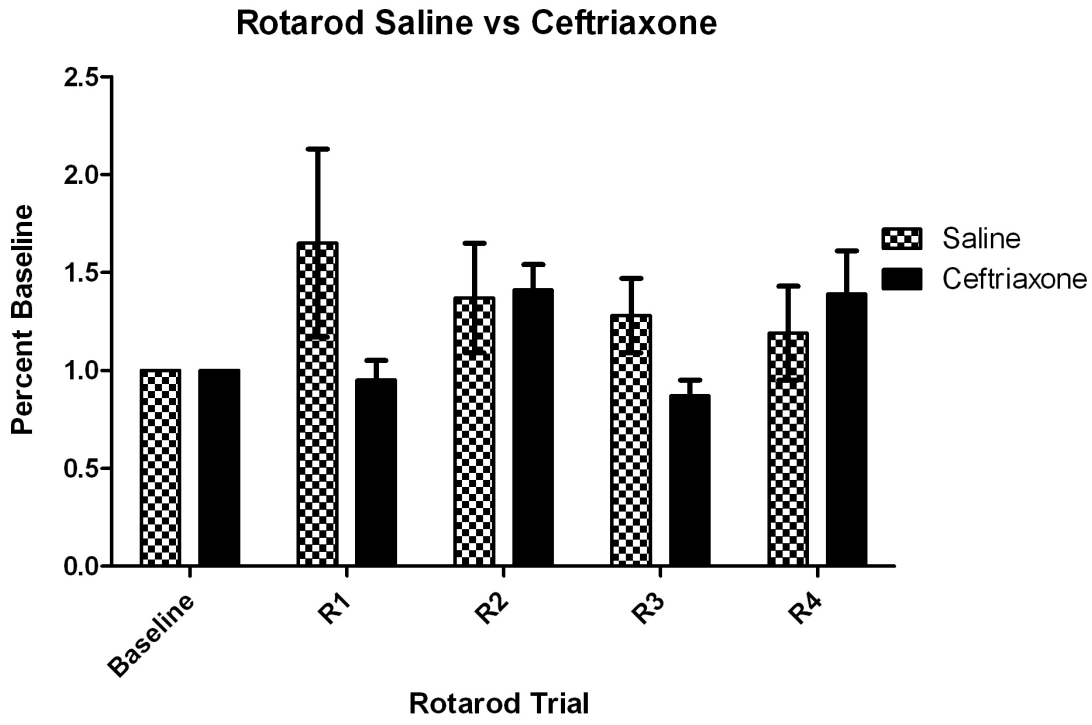

Figure 3.7 Bi-weekly rotarod motor performance of male R6/2 mice treated for 14 days with 100 mg/kg (i.p.) Ceftriaxone (n=15), or saline (n=10). Graph represents average rotarod performance expressed in percent baseline (±SEM) one day prior to treatment and during the treatment (days 1-14). T-test analyses revealed no significance difference among control and treatments group.

A repeated measures ANOVA revealed a main effect of time, $F(1, 4) = 2.918$, $p < 0.05$, when comparing rotarod trials within the Ceftriaxone and saline treated groups and an interaction (treatment X time) effect, $F(1, 4) = 3.593$, $p < 0.05$.

3.3 Sulforaphane Groups

3.3.1 Effect of Sulforaphane treatment on motor performance

Rotarod motor performance test was measured before and during treatment with Sulforaphane to determine if treatment attenuated motor deficiencies in the HD mouse model. The rotarod trials were measured in seconds and normalized to a percent baseline number to account for variability from subject to subject. Baseline (B) motor tests were taken one day prior to beginning treatment. All subsequent rotarod trials are denoted using R1, 2, 3, and 4, as they represent a range of days. Figure 3.8 shows the average rotarod performance by group, SULF 5, and SULF 10 compared to saline treated, plotted against percent baseline. The treatment groups were: saline (n=10), SULF 5mg/kg (n=4), and SULF 10mg/kg (n=9).

Differences in motor performance were evaluated using two-way ANOVA (time X treatment), followed by a posthoc Dunnett multiple comparisons test. SULF 10 treatment decreased rotarod performance, $F(2,8)=3.805$, $p<0.05$.

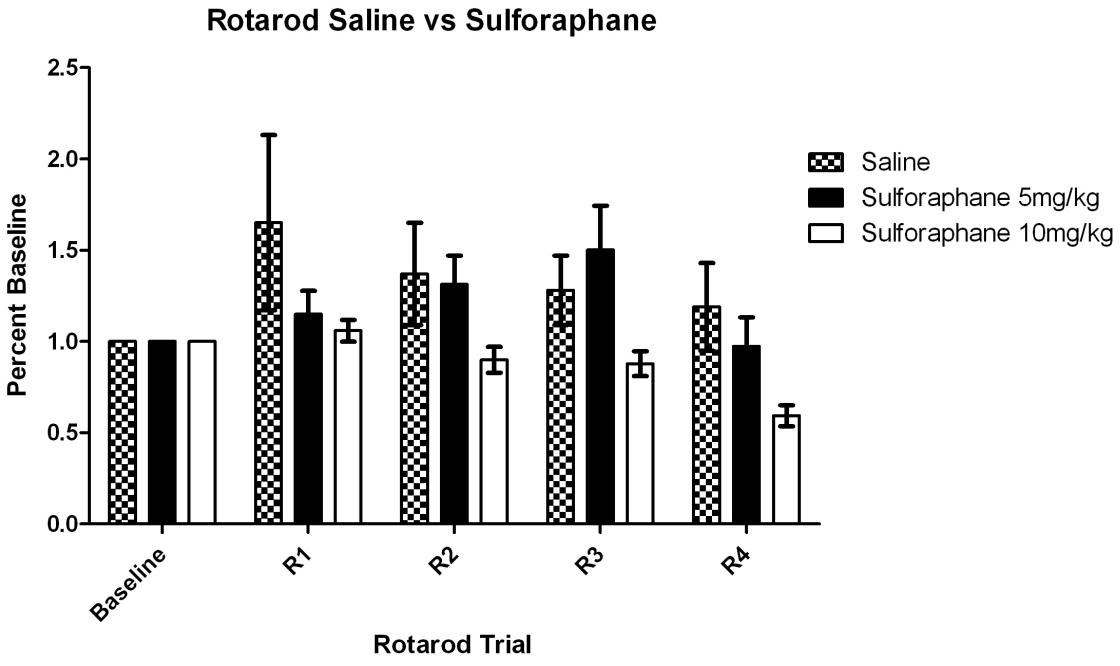

Figure 3.8 Bi-weekly rotarod motor performance of male R6/2 mice treated for 14 days with 5 mg/kg (i.p.) Sulforaphane (n=4), 10 mg/kg (i.p.) Sulforaphane (n=9), or saline (n=11). Graph represents average rotarod performance expressed in percent baseline (±SEM) one day prior to treatment and during the treatment (days 1-14). Two-way ANOVA analyses revealed SULF 10 treatment decreased rotarod performance, $F(2,8)=3.805$, $p<0.05$.

A repeated measures ANOVA revealed a main effect of time, $F(1, 4) =2.77$, $p<0.05$, when comparing rotarod trials within the Sulforaphane groups and saline treated group.

3.4 Effect of Treatments on Body Weight

Weight of each animal was taken before and during treatment with each drug group, as well as with saline. We wanted to monitor whether treatment affected the body weight of the animal for two reasons; first, if the drug was toxic and caused the animals

to lose weight. Secondly, weight loss is common with HD patients and monitoring for body weight was another possible measure of improvement by treatment. A one-way ANOVA was performed, followed by a posthoc Dunnett multiple comparisons test, which revealed no significant difference in body weight among treatment groups [$p>0.05$], or groups by day (Figure 3.9).

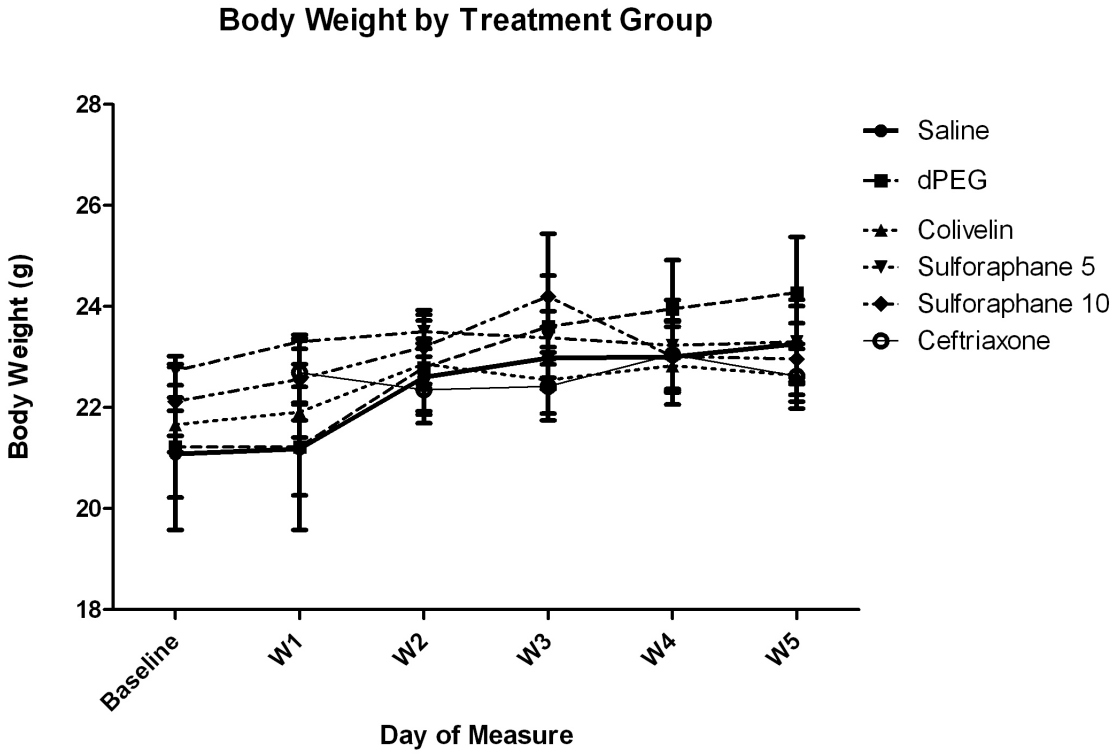

Figure 3.9 Bi-weekly body weight of male R6/2 mice treated for 14 days with 100 mg/kg (i.p.) Ceftriaxone (n=15), 5 mg/kg (i.p.) Sulforaphane (n=4), 10 mg/kg (i.p.) Sulforaphane (n=9), 40 mg/20g (i.p.) Colivelin (n=8), 40 mg/20g (i.p.) dPEG-Colivelin (n=11), or saline (n=11). Graph represents average weight (±SEM) in grams one day prior to treatment and during the treatment (days 1-14). No treatment affected the body weight across the 14 days.

Chapter 4

Discussion

The mice in this study were obtained within the fifth week of age and treatment began during the sixth week of age. During that initial week the animals were acclimated to the vivarium and baseline motor behavior was evaluated. All further motor tests would be evaluated against this initial baseline trial to give us the percent baseline values, demonstrating the improvement or decline in the animals' motor capabilities. The trials are represented by trial number (R1-R4) as they were performed within a range of days within the 14 day treatment period The motor tests were separated by an average of four treatment days, but a minimum of two days, and the last motor test was always evaluated on the last day of treatment to evaluate the performance of a fourteen day course of treatment.

4.1 Colivelin and dPEG-Colivelin Groups

Although Colivelin and dPEG Colivelin treatments did not show any motor performance improvement, we found that Colivelin treatment increased the level of *p*-Akt, which is part of the pro-survival signaling pathway. Studies report that phosphorylation of huntingtin (htt) by Akt kinase can lead to increased BDNF vesicle transport, improving viability of striatal neurons, namely medium spiny neurons that are greatly affected in HD (Cattaneo, Zuccato et al. 2005; Colin, Zala et al. 2008). The increase in the level of *p*-Akt in the ST in animals treated with Colivelin, suggests there might be potential therapeutic effect of this drug for HD.

We also tested dPEG-Colivelin in R6/2 mice. Similarly to Colivelin, we did not observe behavioral motor improvement as compared to saline treated mice. In addition, we did not observe significance in the level of *p*-Akt as compared to the saline treated groups. The addition of the dPEG fragment to Colivelin may improve the stability and biodistribution of this drug into the CNS (Veronese and Mero 2008). Unfortunately, we did not see beneficial effect with the addition of dPEG.

Colivelin treatment did not help to inhibit JNK, producing significant up-regulation of *p*-JNK in both the PFC and ST, when compared to saline treated animals. JNK, part of the mitogen-activated protein kinases (MAPK) signaling pathway, is known to be associated with neurotoxicity in HD (Trotti, Danbolt et al. 1998; Shinkai, Sumi et al. 2006; Perrin, Dufour et al. 2009; Reijonen, Kukkonen et al. 2010). This could attribute to the decreased motor function of the Colivelin treated animals, especially with the increased JNK in the ST, possibly affecting the neurodegeneration of striatal medium

spiny neurons. The dPEGylation of the compound Colivelin was not effective in increasing the efficacy of Colivelin itself. We also did not find a significant difference in the level of *p*-JNK as compared to saline treated mice.

The positive up-regulation of *p*-Akt in the ST of Colivelin treated R6/2 mice could be reason enough to test the combination of Colivelin with another drugs in order to explore the possibilities of a multi-faceted treatment option in HD. Compounds such as Sulforaphane could address another aspect of the disease state that Colivelin has no effect on, such as oxidative stress. With such a wide variety of degeneration occurring, the use of more than one drug to treat HD once a diagnosis has been made is a realistic possibility. There may also be improved results when Colivelin is given prior to symptoms arising, i.e. the drug administered earlier than six weeks of age. As we were unable to obtain the animal model any earlier this was not possible at this point, but may be a possibility at a later time.

4.2 Ceftriaxone Groups

Studies using R6/1 and R6/2 HD mouse models have demonstrated that GLT1 down-regulation may be responsible for the decrease in glutamate uptake by glial cells in the striatum, which can impair regulation of synaptic transmission in HD (Lievens, Woodman et al. 2001; Behrens, Franz et al. 2002; Shin, Fang et al. 2005; Miller, Dorner et al. 2008; Estrada-Sanchez, Montiel et al. 2009). It has been shown by our group that R6/2 mice treated with 200 mg/kg Ceftriaxone for five days showed behavioral

improvement associated with reversal of glutamate uptake deficiencies, and increase in expression of GLT1 (Miller, Dorner et al. 2008).

In the present study, Ceftriaxone treatment for 14 days showed positive trends on trial days R2 and R4, but no significance was found using a T-Test comparing motor behavior to the saline treated groups. A repeated measures ANOVA revealed a main effect of time when comparing rotarod trials within the Ceftriaxone and saline treated groups and an interaction (treatment X time) effect. Previous studies using the same mouse model has shown that GLT1 downregulation may be responsible for the decrease in glutamate uptake by glial cells in the striatum (Lievens, Woodman et al. 2001; Behrens, Franz et al. 2002; Shin, Fang et al. 2005; Miller, Dorner et al. 2008; Estrada-Sanchez, Montiel et al. 2009). It is noteworthy that Ceftriaxone has potential to increase uptake that is mediated through upregulation of GLT1. Reduced extracellular glutamate may have an impact in reduced neurotoxicity.

Treating the animals every day for 14 days was the main reason behind using the lower 100 mg/kg dose of Ceftriaxone in this study. Although the drug showed effectiveness at 200 mg/kg in pervious studies, we wanted to explore the possibility of a lower dose being effective over a longer course of treatment. The lack of consistent improvement at this dosage begs the question whether 100 mg/kg of Ceftriaxone is sub-therapeutic. Increasing the dose of Ceftriaxone to 200 mg/kg will help to evaluate the capability of the drug to improve motor performance across the entire trial instead of only improving selective days. An additional dose and the evaluation of the signaling pathways could help this study to strengthen the profile of Ceftriaxone as a possible treatment for HD.

4.3 Sulforaphane Groups

Sulforaphane, a naturally occurring compound found in broccoli, has shown to be protective against oxidative stress by inducing Phase II enzymes, decreasing degradation of Nrf-2, and exert neuroprotection (Zhang, Talalay et al. 1992; Zhang, Lo et al. 2004; Shinkai, Sumi et al. 2006). Other studies have also shown Sulforaphane protects against neuronal cell death (Kraft, Johnson et al. 2004; Danilov, Chandrasekaran et al. 2009; Tarozzi, Morroni et al. 2009; Soane, Dai et al. 2010); therefore, we chose to test Sulforaphane as a therapeutic, anti-oxidant to protect against neurodegeneration in HD.

Of the two doses of Sulforaphane, the 5mg/kg (SULF 5) dose showed a trend toward potential improved of motor behavior in R6/2 mice except during the final trial. We are hopeful that the antioxidant properties of SULF 5 were able to prevent neurotoxicity as a result of oxidative stress. Further studies need to be performed to identify the signaling pathway of the treatment, as well as a repeated study to increase the subjects in the treatment study. Sulforaphane 10mg/kg (SULF 10) resulted in motor behavior less than those without treatment. SULF 10 may produce toxic effects in a dose-dependent manner.

If administered prior to inclusion formation in the brain, or before symptoms occur, the anti-oxidant properties of Sulforaphane may have a greater ability to control damage as a result of oxidative stress. In the future, Sulforaphane should be studied at an earlier stage in the disease to see if the effect is improved. We should also explore the

long-term effects of Sulforaphane to see if the compound could improve longevity of the HD animal model.

4.4 Limitations to Study

Using the R6/2 animal model helped the study to evaluate the cause-and-effect relationships between treatment and motor behavior, but this mouse model also imposed many difficulties. Then animal subjects began treatment beginning week six due to the ability to procure the animals prior to week five. Three days acclimation to the vivarium, and a baseline motor test put the beginning of injections at six weeks of age. Since symptoms begin to arise no later than week six, we were unable to begin treatment prior to expression of symptoms. Preferably, we would have begun treatment before the development of symptoms or inclusions in the brain, which develop as early as 14 days of age. The R6/2 mouse model is particularly difficult to breed; another obstacle in starting earlier treatment.

In contrast, studying such a young animal questions the ability of the mouse to perform the behavioral rotarod test. The initial baseline evaluation is taken one day prior the administration of treatment and the animal may underperform due to lack of exposure to the rotarod, as well as learn to perform better the next trial. We attempted to control for learning by setting longer intervals between tests, but we cannot say that the animals did not learn before they began to degenerate. We also could further evaluate the ability of the wild type R6/2 mice, both treated and untreated, to clarify any ability to learn or whether the drug itself improved the ability of the mouse to perform the motor tasks.

5. Conclusion

Although we did not observe any behavioral motor improvement with Colivelin, we did, in fact, find significant up-regulation of *p*-Akt in the ST of 40 mg/20g Colivelin treated R6/2 mice as compared to saline treated R6/2 mice. However, we did not observe any significant difference in the level of *p*-Akt in the PFC with Colivelin treatment as compared to saline treated groups. Moreover, significant up-regulation of JNK was observed in the ST and PFC of 40 mg/20g Colivelin treated R6/2 mice. We did not observe any significant difference in the motor behavioral performances in dPEG-Colivelin, Ceftriaxone, or Sulforaphane treated R6/2 mice.

6. References

Aiken, C. T., A. J. Tobin, et al. (2004). "A cell-based screen for drugs to treat Huntington's disease." Neurobiol Dis **16**(3): 546-555.

Alberch, J., E. Perez-Navarro, et al. (2004). "Neurotrophic factors in Huntington's disease." Prog Brain Res **146**: 195-229.

Albin, R. L., A. B. Young, et al. (1990). "Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease." N Engl J Med **322**(18): 1293-1298.

Alcantara, S., J. Frisen, et al. (1997). "TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death." J Neurosci **17**(10): 3623-3633.

Anderson, K. D., N. Panayotatos, et al. (1996). "Ciliary neurotrophic factor protects striatal output neurons in an animal model of Huntington disease." Proc Natl Acad Sci U S A **93**(14): 7346-7351.

Andrew, S. E., Y. P. Goldberg, et al. (1993). "The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease." Nat Genet **4**(4): 398-403.

Appel, S. H. (1981). "A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer disease." Ann Neurol **10**(6): 499-505.

Asher, S. W. and M. J. Aminoff (1981). "Tetrabenazine and movement disorders." Neurology **31**(8): 1051-1054.

Aziz, N. A., C. K. Jurgens, et al. (2009). "Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease." Neurology **73**(16): 1280-1285.

Backman, L., T. B. Robins-Wahlin, et al. (1997). "Cognitive deficits in Huntington's disease are predicted by dopaminergic PET markers and brain volumes." Brain **120 (Pt 12)**: 2207-2217.

Bamford, N. S., S. Robinson, et al. (2004). "Dopamine modulates release from corticostriatal terminals." J Neurosci **24**(43): 9541-9552.

Barr, A. N., J. H. Fischer, et al. (1988). "Serum haloperidol concentration and choreiform movements in Huntington's disease." Neurology **38**(1): 84-88.

Bassan, M., R. Zamostiano, et al. (1999). "Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide." J Neurochem **72**(3): 1283-1293.

Bates, G. (2003). "Huntingtin aggregation and toxicity in Huntington's disease." Lancet **361**(9369): 1642-1644.

Bates, G., P. S. Harper, et al. (2002). Huntington's disease. Oxford ; New York, Oxford University Press.

Beglinger, L. J., P. C. Nopoulos, et al. (2005). "White matter volume and cognitive dysfunction in early Huntington's disease." Cogn Behav Neurol **18**(2): 102-107.

Behrens, P. F., P. Franz, et al. (2002). "Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation." Brain **125**(Pt 8): 1908-1922.

Bergerot, A., P. J. Shortland, et al. (2004). "Co-treatment with riluzole and GDNF is necessary for functional recovery after ventral root avulsion injury." Exp Neurol **187**(2): 359-366.

Bezprozvanny, I. and M. R. Hayden (2004). "Deranged neuronal calcium signaling and Huntington disease." Biochem Biophys Res Commun **322**(4): 1310-1317.

Bibb, J. A., Z. Yan, et al. (2000). "Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice." Proc Natl Acad Sci U S A **97**(12): 6809-6814.

Bloch, J., A. C. Bachoud-Levi, et al. (2004). "Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study." Hum Gene Ther **15**(10): 968-975.

Blondel, O., C. Collin, et al. (2000). "A glia-derived signal regulating neuronal differentiation." J Neurosci **20**(21): 8012-8020.

Bonelli, R. M. and M. F. Beal (2012). "Huntington's disease." Handb Clin Neurol **106**: 507-526.

Bonelli, R. M., C. Heuberger, et al. (2003). "Minocycline for Huntington's disease: an open label study." Neurology **60**(5): 883-884.

Bonelli, R. M. and P. Hofmann (2007). "A systematic review of the treatment studies in Huntington's disease since 1990." Expert Opin Pharmacother **8**(2): 141-153.

Brandt, J., F. W. Bylsma, et al. (1995). "Impaired source memory in Huntington's disease and its relation to basal ganglia atrophy." J Clin Exp Neuropsychol **17**(6): 868-877.

Brenneman, D. E. and I. Gozes (1996). "A femtomolar-acting neuroprotective peptide." J Clin Invest **97**(10): 2299-2307.

Brenneman, D. E., J. Hauser, et al. (1998). "Activity-dependent neurotrophic factor: structure-activity relationships of femtomolar-acting peptides." J Pharmacol Exp Ther **285**(2): 619-627.

Brouillet, E., F. Conde, et al. (1999). "Replicating Huntington's disease phenotype in experimental animals." Prog Neurobiol **59**(5): 427-468.

Burgunder, J. M., M. Guttman, et al. (2011). "An International Survey-based Algorithm for the Pharmacologic Treatment of Chorea in Huntington's Disease." PLoS Curr **3**: RRN1260.

Calkins, M. J., D. A. Johnson, et al. (2009). "The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease." Antioxid Redox Signal **11**(3): 497-508.

Cattaneo, E., D. Rigamonti, et al. (2001). "Loss of normal huntingtin function: new developments in Huntington's disease research." Trends Neurosci **24**(3): 182-188.

Cattaneo, E., C. Zuccato, et al. (2005). "Normal huntingtin function: an alternative approach to Huntington's disease." Nat Rev Neurosci **6**(12): 919-930.

Cha, J. H. (2007). "Transcriptional signatures in Huntington's disease." Prog Neurobiol **83**(4): 228-248.

Chiba, T., Y. Hashimoto, et al. (2004). "Neuroprotective effect of activity-dependent neurotrophic factor against toxicity from familial amyotrophic lateral sclerosis-linked mutant SOD1 in vitro and in vivo." J Neurosci Res **78**(4): 542-552.

Chiba, T., I. Nishimoto, et al. (2007). "Neuroprotection against neurodegenerative diseases: development of a novel hybrid neuroprotective peptide Colivelin." Mol Neurobiol **35**(1): 55-84.

Chiba, T., M. Yamada, et al. (2009). "Targeting the JAK2/STAT3 axis in Alzheimer's disease." Expert Opin Ther Targets **13**(10): 1155-1167.

Chiba, T., M. Yamada, et al. (2005). "Development of a femtomolar-acting humanin derivative named colivelin by attaching activity-dependent neurotrophic factor to its N terminus: characterization of colivelin-mediated neuroprotection against Alzheimer's disease-relevant insults in vitro and in vivo." J Neurosci **25**(44): 10252-10261.

Chiba, T., M. Yamada, et al. (2006). "Colivelin prolongs survival of an ALS model mouse." Biochem Biophys Res Commun **343**(3): 793-798.

Chiba, T., M. Yamada, et al. (2009). "Amyloid-beta causes memory impairment by disturbing the JAK2/STAT3 axis in hippocampal neurons." Mol Psychiatry **14**(2): 206-222.

Choo, Y. S., G. V. Johnson, et al. (2004). "Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release." Hum Mol Genet **13**(14): 1407-1420.

Colin, E., D. Zala, et al. (2008). "Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons." EMBO J **27**(15): 2124-2134.

Connor, B. and M. Dragunow (1998). "The role of neuronal growth factors in neurodegenerative disorders of the human brain." Brain Res Brain Res Rev **27**(1): 1-39.

Cornett, J., F. Cao, et al. (2005). "Polyglutamine expansion of huntingtin impairs its nuclear export." Nat Genet **37**(2): 198-204.

Coyle, J. T. and P. Puttfarcken (1993). "Oxidative stress, glutamate, and neurodegenerative disorders." Science **262**(5134): 689-695.

Danilov, C. A., K. Chandrasekaran, et al. (2009). "Sulforaphane protects astrocytes against oxidative stress and delayed death caused by oxygen and glucose deprivation." Glia **57**(6): 645-656.

de la Monte, S. M., J. P. Vonsattel, et al. (1988). "Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease." J Neuropathol Exp Neurol **47**(5): 516-525.

de Tommaso, M., N. Specchio, et al. (2004). "Effects of rivastigmine on motor and cognitive impairment in Huntington's disease." Mov Disord **19**(12): 1516-1518.

Deister, C. and C. E. Schmidt (2006). "Optimizing neurotrophic factor combinations for neurite outgrowth." J Neural Eng **3**(2): 172-179.

DiFiglia, M. (1990). "Excitotoxic injury of the neostriatum: a model for Huntington's disease." Trends Neurosci **13**(7): 286-289.

DiFiglia, M., E. Sapp, et al. (1995). "Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons." Neuron **14**(5): 1075-1081.

Dong, X. X., Y. Wang, et al. (2009). "Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases." Acta Pharmacol Sin **30**(4): 379-387.

Dure, L. S. t., A. B. Young, et al. (1991). "Excitatory amino acid binding sites in the caudate nucleus and frontal cortex of Huntington's disease." Ann Neurol **30**(6): 785-793.

Dyer, R. B. and C. T. McMurray (2001). "Mutant protein in Huntington disease is resistant to proteolysis in affected brain." Nat Genet **29**(3): 270-278.

Emerich, D. F., S. R. Winn, et al. (1997). "Protective effect of encapsulated cells producing neurotrophic factor CNTF in a monkey model of Huntington's disease." Nature **386**(6623): 395-399.

Estrada-Sanchez, A. M., T. Montiel, et al. (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." Neurobiol Dis **34**(1): 78-86.

Evans, D. L., C. A. Pedersen, et al. (1987). "ECT in the Treatment of Organic Psychosis in Huntington's Disease." Convuls Ther **3**(2): 145-150.

Everett, C. M. and N. W. Wood (2004). "Trinucleotide repeats and neurodegenerative disease." Brain **127**(Pt 11): 2385-2405.

Fernandez, H. H., J. H. Friedman, et al. (2000). "Donepezil for Huntington's disease." Mov Disord **15**(1): 173-176.

Ferrante, R. J., C. A. Gutekunst, et al. (1997). "Heterogeneous topographic and cellular distribution of huntingtin expression in the normal human neostriatum." J Neurosci **17**(9): 3052-3063.

Ferrante, R. J., N. W. Kowall, et al. (1987). "Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease." J Neuropathol Exp Neurol **46**(1): 12-27.

Frank, S. and J. Jankovic (2010). "Advances in the pharmacological management of Huntington's disease." Drugs **70**(5): 561-571.

Gervais, F. G., R. Singaraja, et al. (2002). "Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi." Nat Cell Biol **4**(2): 95-105.

Gibson, H. E., K. Reim, et al. (2005). "A similar impairment in CA3 mossy fibre LTP in the R6/2 mouse model of Huntington's disease and in the complexin II knockout mouse." Eur J Neurosci **22**(7): 1701-1712.

Gimenez-Roldan, S. and D. Mateo (1989). "[Huntington disease: tetrabenazine compared to haloperidol in the reduction of involuntary movements]." Neurologia **4**(8): 282-287.

Ginovart, N., A. Lundin, et al. (1997). "PET study of the pre- and post-synaptic dopaminergic markers for the neurodegenerative process in Huntington's disease." Brain **120 (Pt 3)**: 503-514.

Glass, D. J. and G. D. Yancopoulos (1993). "The neurotrophins and their receptors." Trends Cell Biol **3**(8): 262-268.

Glazner, G. W., A. Boland, et al. (1999). "Activity-dependent neurotrophic factor peptide (ADNF9) protects neurons against oxidative stress-induced death." J Neurochem **73**(6): 2341-2347.

Goehler, H., M. Lalowski, et al. (2004). "A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease." Mol Cell **15**(6): 853-865.

Goldberg, Y. P., D. W. Nicholson, et al. (1996). "Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract." Nat Genet **13**(4): 442-449.

Gong, B., C. Kielar, et al. (2012). "Temporal separation of aggregation and ubiquitination during early inclusion formation in transgenic mice carrying the Huntington's disease mutation." PLoS One **7**(7): e41450.

Graham, R. K., Y. Deng, et al. (2006). "Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin." Cell **125**(6): 1179-1191.

Hackam, A. S., A. S. Yassa, et al. (2000). "Huntingtin interacting protein 1 induces apoptosis via a novel caspase-dependent death effector domain." J Biol Chem **275**(52): 41299-41308.

Halliday, G. M., D. A. McRitchie, et al. (1998). "Regional specificity of brain atrophy in Huntington's disease." Exp Neurol **154**(2): 663-672.

Hamanoue, M., N. Takemoto, et al. (1996). "Neurotrophic effect of hepatocyte growth factor on central nervous system neurons in vitro." J Neurosci Res **43**(5): 554-564.

Haque, N. S. and O. Isacson (2000). "Neurotrophic factors NGF and FGF-2 alter levels of huntingtin (IT15) in striatal neuronal cell cultures." Cell Transplant **9**(5): 623-627.

Harjes, P. and E. E. Wanker (2003). "The hunt for huntingtin function: interaction partners tell many different stories." Trends Biochem Sci **28**(8): 425-433.

Hashimoto, Y., T. Niikura, et al. (2001). "Detailed characterization of neuroprotection by a rescue factor humanin against various Alzheimer's disease-relevant insults." J Neurosci **21**(23): 9235-9245.

Hedreen, J. C. and S. E. Folstein (1995). "Early loss of neostriatal striosome neurons in Huntington's disease." J Neuropathol Exp Neurol **54**(1): 105-120.

Hickey, M. A. and M. F. Chesselet (2003). "Apoptosis in Huntington's disease." Prog Neuropsychopharmacol Biol Psychiatry **27**(2): 255-265.

Hilditch-Maguire, P., F. Trettel, et al. (2000). "Huntingtin: an iron-regulated protein essential for normal nuclear and perinuclear organelles." Hum Mol Genet **9**(19): 2789-2797.

Hodgson, J. G., N. Agopyan, et al. (1999). "A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration." Neuron **23**(1): 181-192.

Hoffner, G., P. Kahlem, et al. (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**(Pt 5): 941-948.

Humbert, S., E. A. Bryson, et al. (2002). "The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt." Dev Cell **2**(6): 831-837.

Huntington, G. (2003). "On chorea. George Huntington, M.D." J Neuropsychiatry Clin Neurosci **15**(1): 109-112.

Hurlbert, M. S., W. Zhou, et al. (1999). "Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes." Diabetes **48**(3): 649-651.

Jankovic, J. and J. Beach (1997). "Long-term effects of tetrabenazine in hyperkinetic movement disorders." Neurology **48**(2): 358-362.

Jernigan, T. L., D. P. Salmon, et al. (1991). "Cerebral structure on MRI, Part II: Specific changes in Alzheimer's and Huntington's diseases." Biol Psychiatry **29**(1): 68-81.

Jin, K., M. LaFevre-Bernt, et al. (2005). "FGF-2 promotes neurogenesis and neuroprotection and prolongs survival in a transgenic mouse model of Huntington's disease." Proc Natl Acad Sci U S A **102**(50): 18189-18194.

Kassubek, J., F. D. Juengling, et al. (2005). "Thalamic atrophy in Huntington's disease co-varies with cognitive performance: a morphometric MRI analysis." Cereb Cortex **15**(6): 846-853.

Katoh-Semba, R., T. Asano, et al. (2002). "Riluzole enhances expression of brain-derived neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus." FASEB J **16**(10): 1328-1330.

Kegel, K. B., A. R. Meloni, et al. (2002). "Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription." J Biol Chem **277**(9): 7466-7476.

Kraft, A. D., D. A. Johnson, et al. (2004). "Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult." Journal of Neuroscience **24**(5): 1101-1112.

Kruttgen, A., S. Saxena, et al. (2003). "Neurotrophins and neurodegenerative diseases: receptors stuck in traffic?" J Neuropathol Exp Neurol **62**(4): 340-350.

Kumar, P., H. Kalonia, et al. (2010). "Huntington's disease: pathogenesis to animal models." Pharmacol Rep **62**(1): 1-14.

Laforet, G. A., E. Sapp, et al. (2001). "Changes in cortical and striatal neurons predict behavioral and electrophysiological abnormalities in a transgenic murine model of Huntington's disease." J Neurosci **21**(23): 9112-9123.

Leavitt, B. R., J. A. Guttman, et al. (2001). "Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo." Am J Hum Genet **68**(2): 313-324.

Leavitt, B. R., J. M. van Raamsdonk, et al. (2006). "Wild-type huntingtin protects neurons from excitotoxicity." J Neurochem **96**(4): 1121-1129.

Levine, M. S., C. Cepeda, et al. (2004). "Genetic mouse models of Huntington's and Parkinson's diseases: illuminating but imperfect." Trends Neurosci **27**(11): 691-697.

Li, J. Y., M. Plomann, et al. (2003). "Huntington's disease: a synaptopathy?" Trends Mol Med **9**(10): 414-420.

Li, S. H. and X. J. Li (2004). "Huntingtin-protein interactions and the pathogenesis of Huntington's disease." Trends Genet **20**(3): 146-154.

Lievens, J. C., B. Woodman, et al. (2001). "Impaired glutamate uptake in the R6 Huntington's disease transgenic mice." Neurobiol Dis **8**(5): 807-821.

Linker, R. A., M. Maurer, et al. (2002). "CNTF is a major protective factor in demyelinating CNS disease: a neurotrophic cytokine as modulator in neuroinflammation." Nat Med **8**(6): 620-624.

London, E. D., H. I. Yamamura, et al. (1981). "Decreased receptor-binding sites for kainic acid in brains of patients with Huntington's disease." Biol Psychiatry **16**(2): 155-162.

Mangiarini, L., K. Sathasivam, et al. (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**(3): 493-506.

Matsuoka, M., Y. Hashimoto, et al. (2006). "Humanin and colivelin: neuronal-death-suppressing peptides for Alzheimer's disease and amyotrophic lateral sclerosis." CNS Drug Rev **12**(2): 113-122.

Mehvar, R. and F. Jamali (1987). "Concentration-effect relationships of tetrabenazine and dihydrotetrabenazine in the rat." J Pharm Sci **76**(6): 461-465.

Menalled, L. B. and M. F. Chesselet (2002). "Mouse models of Huntington's disease." Trends Pharmacol Sci **23**(1): 32-39.

Milakovic, T., R. A. Quintanilla, et al. (2006). "Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells: functional consequences." J Biol Chem **281**(46): 34785-34795.

Miller, B. R., J. L. Dorner, et al. (2008). "Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse." Neuroscience **153**(1): 329-337.

Morton, A. J., M. A. Lagan, et al. (2000). "Progressive formation of inclusions in the striatum and hippocampus of mice transgenic for the human Huntington's disease mutation." J Neurocytol **29**(9): 679-702.

Murer, M. G., Q. Yan, et al. (2001). "Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease." Prog Neurobiol **63**(1): 71-124.

Nakao, N., P. Brundin, et al. (1995). "Trophic and protective actions of brain-derived neurotrophic factor on striatal DARPP-32-containing neurons in vitro." Brain Res Dev Brain Res **90**(1-2): 92-101.

Ooi, L. and I. C. Wood (2007). "Chromatin crosstalk in development and disease: lessons from REST." Nat Rev Genet **8**(7): 544-554.

Oyanagi, K., S. Takeda, et al. (1989). "A quantitative investigation of the substantia nigra in Huntington's disease." Ann Neurol **26**(1): 13-19.

Paleacu, D., M. Anca, et al. (2002). "Olanzapine in Huntington's disease." Acta Neurol Scand **105**(6): 441-444.

Pascual, M. and C. Guerri (2007). "The peptide NAP promotes neuronal growth and differentiation through extracellular signal-regulated protein kinase and Akt pathways, and protects neurons co-cultured with astrocytes damaged by ethanol." J Neurochem **103**(2): 557-568.

Paulsen, J. S., D. R. Langbehn, et al. (2008). "Detection of Huntington's disease decades before diagnosis: the Predict-HD study." J Neurol Neurosurg Psychiatry **79**(8): 874-880.

Peinemann, A., S. Schuller, et al. (2005). "Executive dysfunction in early stages of Huntington's disease is associated with striatal and insular atrophy: a neuropsychological and voxel-based morphometric study." J Neurol Sci **239**(1): 11-19.

Perez-Navarro, E., E. Arenas, et al. (1996). "Glial cell line-derived neurotrophic factor protects striatal calbindin-immunoreactive neurons from excitotoxic damage." Neuroscience **75**(2): 345-352.

Perrin, V., N. Dufour, et al. (2009). "Implication of the JNK pathway in a rat model of Huntington's disease." Exp Neurol **215**(1): 191-200.

Perutz, M. F., T. Johnson, et al. (1994). "Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases." Proc Natl Acad Sci U S A **91**(12): 5355-5358.

Pillai, J. A., L. A. Hansen, et al. (2012). "Clinical severity of Huntington's disease does not always correlate with neuropathologic stage." Mov Disord **27**(9): 1099-1103.

Politis, M., N. Pavese, et al. (2008). "Hypothalamic involvement in Huntington's disease: an in vivo PET study." Brain **131**(Pt 11): 2860-2869.

Popovic, N., M. Maingay, et al. (2005). "Lentiviral gene delivery of GDNF into the striatum of R6/2 Huntington mice fails to attenuate behavioral and neuropathological changes." Exp Neurol **193**(1): 65-74.

Pringsheim, T., K. Wiltshire, et al. (2012). "The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis." Mov Disord **27**(9): 1083-1091.

Puri, B. K., G. M. Bydder, et al. (2002). "MRI and neuropsychological improvement in Huntington disease following ethyl-EPA treatment." Neuroreport **13**(1): 123-126.

Rangone, H., G. Poizat, et al. (2004). "The serum- and glucocorticoid-induced kinase SGK inhibits mutant huntingtin-induced toxicity by phosphorylating serine 421 of huntingtin." Eur J Neurosci **19**(2): 273-279.

Reijonen, S., J. P. Kukkonen, et al. (2010). "Downregulation of NF-kappaB signaling by mutant huntingtin proteins induces oxidative stress and cell death." Cell Mol Life Sci **67**(11): 1929-1941.

Reiner, A., R. L. Albin, et al. (1988). "Differential loss of striatal projection neurons in Huntington disease." Proc Natl Acad Sci U S A **85**(15): 5733-5737.

Reiner, A., N. Del Mar, et al. (2001). "Neurons lacking huntingtin differentially colonize brain and survive in chimeric mice." J Neurosci **21**(19): 7608-7619.

Rigamonti, D., J. H. Bauer, et al. (2000). "Wild-type huntingtin protects from apoptosis upstream of caspase-3." J Neurosci **20**(10): 3705-3713.

Rigamonti, D., S. Sipione, et al. (2001). "Huntingtin's neuroprotective activity occurs via inhibition of procaspase-9 processing." J Biol Chem **276**(18): 14545-14548.

Rosas, H. D., D. S. Tuch, et al. (2006). "Diffusion tensor imaging in presymptomatic and early Huntington's disease: Selective white matter pathology and its relationship to clinical measures." Mov Disord **21**(9): 1317-1325.

Rosenblatt, A. (2007). "Neuropsychiatry of Huntington's disease." Dialogues Clin Neurosci **9**(2): 191-197.

Ross, C. A. (2004). "Huntington's disease: new paths to pathogenesis." Cell **118**(1): 4-7.

Roth, J., J. Klempii, et al. (2005). "Caudate nucleus atrophy in Huntington's disease and its relationship with clinical and genetic parameters." Funct Neurol **20**(3): 127-130.

Rubinsztein, D. C. (2006). "The roles of intracellular protein-degradation pathways in neurodegeneration." Nature **443**(7113): 780-786.

Sari, Y. (2011). "Huntington's Disease: From Mutant Huntingtin Protein to Neurotrophic Factor Therapy." Int J Biomed Sci **7**(2): 89-100.

Sari, Y. (2011). "Potential drugs and methods for preventing or delaying the progression of Huntington's disease." Recent Pat CNS Drug Discov **6**(2): 80-90.

Sari, Y., A. L. Prieto, et al. (2010). "Ceftriaxone-induced up-regulation of cortical and striatal GLT1 in the R6/2 model of Huntington's disease." J Biomed Sci **17**: 62.

Seppi, K., J. Mueller, et al. (2001). "Riluzole in Huntington's disease (HD): an open label study with one year follow up." J Neurol **248**(10): 866-869.

Shang, H., A. Danek, et al. (2012). "Huntington's disease: new aspects on phenotype and genotype." Parkinsonism Relat Disord **18 Suppl 1**: S107-109.

Shin, J. Y., Z. H. Fang, et al. (2005). "Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity." J Cell Biol **171**(6): 1001-1012.

Shinkai, Y., D. Sumi, et al. (2006). "Sulforaphane, an activator of Nrf2, suppresses cellular accumulation of arsenic and its cytotoxicity in primary mouse hepatocytes." FEBS Lett **580**(7): 1771-1774.

Simmons, D. A., R. A. Mehta, et al. (2011). "Brief ampakine treatments slow the progression of Huntington's disease phenotypes in R6/2 mice." Neurobiol Dis **41**(2): 436-444.

Soane, L., W. L. Dai, et al. (2010). "Sulforaphane Protects Immature Hippocampal Neurons Against Death Caused by Exposure to Hemin or to Oxygen and Glucose Deprivation." J Neurosci Res **88**(6): 1355-1363.

Sponne, I., A. Fifre, et al. (2004). "Humanin rescues cortical neurons from prion-peptide-induced apoptosis." Mol Cell Neurosci **25**(1): 95-102.

Squitieri, F., A. Ciammola, et al. (2008). "Neuroprotective effects of riluzole in Huntington's disease." Eur J Nucl Med Mol Imaging **35**(1): 221-222.

Squitieri, F., S. Orobello, et al. (2009). "Riluzole protects Huntington disease patients from brain glucose hypometabolism and grey matter volume loss and increases production of neurotrophins." Eur J Nucl Med Mol Imaging **36**(7): 1113-1120.

Stankoff, B., M. S. Aigrot, et al. (2002). "Ciliary neurotrophic factor (CNTF) enhances myelin formation: a novel role for CNTF and CNTF-related molecules." J Neurosci **22**(21): 9221-9227.

Steffan, J. S., L. Bodai, et al. (2001). "Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila." Nature **413**(6857): 739-743.

Suzuki, M., T. J. Desmond, et al. (2001). "Vesicular neurotransmitter transporters in Huntington's disease: initial observations and comparison with traditional synaptic markers." Synapse **41**(4): 329-336.

Tang, T. S., E. Slow, et al. (2005). "Disturbed Ca2+ signaling and apoptosis of medium spiny neurons in Huntington's disease." Proc Natl Acad Sci U S A **102**(7): 2602-2607.

Tang, T. S., H. Tu, et al. (2003). "Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1." Neuron **39**(2): 227-239.

Tarozzi, A., F. Morroni, et al. (2009). "Sulforaphane as an inducer of glutathione prevents oxidative stress-induced cell death in a dopaminergic-like neuroblastoma cell line." J Neurochem **111**(5): 1161-1171.

Terrence, C. F. (1976). "Fluphenazine decanoate in the treatment of chorea: a double-blind study." Curr Ther Res Clin Exp **20**(2): 177-183.

Thakur, A. K., M. Jayaraman, et al. (2009). "Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism." Nat Struct Mol Biol **16**(4): 380-389.

Thibaut, F., B. A. Faucheux, et al. (1995). "Regional distribution of monoamine vesicular uptake sites in the mesencephalon of control subjects and patients with Parkinson's disease: a postmortem study using tritiated tetrabenazine." Brain Res **692**(1-2): 233-243.

Trotti, D., N. C. Danbolt, et al. (1998). "Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration?" Trends Pharmacol Sci **19**(8): 328-334.

Turjanski, N., R. Weeks, et al. (1995). "Striatal D1 and D2 receptor binding in patients with Huntington's disease and other choreas. A PET study." Brain **118 (Pt 3)**: 689-696.

Tzingounis, A. V. and J. I. Wadiche (2007). "Glutamate transporters: confining runaway excitation by shaping synaptic transmission." Nat Rev Neurosci **8**(12): 935-947.

Vaddadi, K. S., E. Soosai, et al. (2002). "A randomised, placebo-controlled, double blind study of treatment of Huntington's disease with unsaturated fatty acids." Neuroreport **13**(1): 29-33.

Velier, J., M. Kim, et al. (1998). "Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways." Exp Neurol **152**(1): 34-40.

Veronese, F. M. and A. Mero (2008). "The impact of PEGylation on biological therapies." BioDrugs **22**(5): 315-329.

Volterra, A., D. Trotti, et al. (1994). "Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes." J Neurosci **14**(5 Pt 1): 2924-2932.

Vonsattel, J. P. and M. DiFiglia (1998). "Huntington disease." J Neuropathol Exp Neurol **57**(5): 369-384.

Wanker, E. E. (2000). "Protein aggregation and pathogenesis of Huntington's disease: mechanisms and correlations." Biol Chem **381**(9-10): 937-942.

Warby, S. C., E. Y. Chan, et al. (2005). "Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo." Hum Mol Genet **14**(11): 1569-1577.

White, J. K., W. Auerbach, et al. (1997). "Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion." Nat Genet **17**(4): 404-410.

Xia, J., D. H. Lee, et al. (2003). "Huntingtin contains a highly conserved nuclear export signal." Hum Mol Genet **12**(12): 1393-1403.

Yamada, M., T. Chiba, et al. (2008). "Nasal Colivelin treatment ameliorates memory impairment related to Alzheimer's disease." Neuropsychopharmacology **33**(8): 2020-2032.

Yohrling, G. J. t., G. C. Jiang, et al. (2003). "Analysis of cellular, transgenic and human models of Huntington's disease reveals tyrosine hydroxylase alterations and substantia nigra neuropathology." Brain Res Mol Brain Res **119**(1): 28-36.

Young, A. B., J. T. Greenamyre, et al. (1988). "NMDA receptor losses in putamen from patients with Huntington's disease." Science **241**(4868): 981-983.

Zeitlin, S., J. P. Liu, et al. (1995). "Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue." Nat Genet **11**(2): 155-163.

Zeron, M. M., H. B. Fernandes, et al. (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**(3): 469-479.

Zhang, D. D., S. C. Lo, et al. (2004). "Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex." Mol Cell Biol **24**(24): 10941-10953.

Zhang, Y., M. Li, et al. (2003). "Depletion of wild-type huntingtin in mouse models of neurologic diseases." J Neurochem **87**(1): 101-106.

Zhang, Y., P. Talalay, et al. (1992). "A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure." Proc Natl Acad Sci U S A **89**(6): 2399-2403.

Zhao, J., N. Kobori, et al. (2006). "Sulforaphane reduces infarct volume following focal cerebral ischemia in rodents." Neurosci Lett **393**(2-3): 108-112.

Zhao, J., A. N. Moore, et al. (2005). "Sulforaphane enhances aquaporin-4 expression and decreases cerebral edema following traumatic brain injury." J Neurosci Res **82**(4): 499-506.

Zhao, J., A. N. Moore, et al. (2007). "Enhancing expression of Nrf2-driven genes protects the blood brain barrier after brain injury." J Neurosci **27**(38): 10240-10248.

Zuccato, C., N. Belyaev, et al. (2007). "Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease." J Neurosci **27**(26): 6972-6983.

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

Zuccato, C., A. Ciammola, et al. (2001). "Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease." Science **293**(5529): 493-498.

Zuccato, C., M. Tartari, et al. (2003). "Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes." Nat Genet **35**(1): 76-83.

Zuccato, C., M. Valenza, et al. (2010). "Molecular mechanisms and potential therapeutical targets in Huntington's disease." Physiol Rev **90**(3): 905-981.