Electroconvulsive Shock Ameliorates Disease Processes And Extends Survival In Huntington Mutant Mice

Akanksha Baharani

University of Central Florida

Part of the Medical Biotechnology Commons

Find similar works at: https://stars.library.ucf.edu/etd

University of Central Florida Libraries http://library.ucf.edu

This Masters Thesis (Open Access) is brought to you for free and open access by STARS. It has been accepted for inclusion in Electronic Theses and Dissertations, 2004-2019 by an authorized administrator of STARS. For more information, please contact STARS@ucf.edu.

STARS Citation
https://stars.library.ucf.edu/etd/1554
ELECTROCONVULSIVE SHOCK AMELIORATES DISEASE PROCESSES AND
EXTENDS SURVIVAL IN HUNTINGTON MUTANT MICE

by

Akanksha Baharani

A dissertation submitted in partial fulfillment of the requirements
for Masters in Biotechnology
in the Burnett school of Biomedical Sciences
in the College of Science
at the University of Central Florida
Orlando, Florida

Summer Term
2010

Major Professor
Dr Sic L Chan, PhD
Huntington's disease (HD) is a devastating autosomal dominantly inherited neurological disorder caused by an abnormal expansion of CAG trinucleotide repeats in the gene coding for the N-terminal region of the huntingtin (Htt) protein, which leads to the formation of a polyglutamine stretch. The greater the CAG repeats, the earlier the onset of the disease. The polyglutamine stretch destabilizes the Htt protein leading to misfolding, abnormal processing, aggregation, and inclusion formation. Mutant Htt protein is believed to damage and kill neurons in the striatum by a mechanism involving increased oxidative and metabolic stress, and impaired adaptive cellular stress responses. A large number of abnormalities have been reported in HD, including transcription deficits, energy impairment, excitotoxicity, and lack of trophic support. Reduced trophic support contributes importantly to striatal degeneration in human HD. Specifically, brain-derived neurotrophic factor (BDNF) expression is reduced in patients with HD. BDNF is also decreased in brain tissue from mice transgenic for mutant Htt. BDNF levels influence the onset and the severity of motor dysfunction in HD mice. In addition to BDNF, levels of the molecular chaperones heat shock proteins (Hsp40 and 70) decrease progressively in HD brain. Hsp70 is a highly stress-inducible member of a chaperone family of proteins that functions to prevent misfolding and aggregation of newly synthesized mutant proteins and stress-denatured proteins. Hsps appear to play a critical role in HD since expression of active heat shock factor HSF1, a transcription factor responsible for the induction of Hsps, markedly reduces polyglutamine aggregate formation in both cell and mouse models.

Many efforts have been made to develop preventive treatments for HD because of the strong genetic link and a freely available genetic test to identify individuals at risk. At present, only symptomatic therapy is available and effective therapeutic approaches to slow the disease
process have yet to be developed. Previous studies have shown that electroconvulsive shock (ECS) induces the production of growth factors including BDNF and the molecular chaperones HSP40 and HSP70. Because ECS can stimulate the production of neuroprotective proteins, we determined whether ECS treatment could slow the progressive nature of the disease process and provide a therapeutic benefit in a mouse model of HD. ECS or sham treatment was administered to male N171-82Q Htt mutant mice. End points measured included motor function, striatal and cortical pathology, and levels of neurotrophic factors, protein chaperones, and proteins involved in synaptic plasticity. ECS treatment delayed the onset of motor symptoms, reduced body weight loss and extended the survival of HD mice. Striatal neurodegeneration was attenuated and levels of neurotrophic factors, protein chaperones and mitochondria-stabilizing protein were elevated in striatal cells of ECS-treated compared to sham-treated HD mice. Our findings suggest that ECS can increase the resistance of neurons to mutant huntingtin resulting in improved functional outcome and extended survival. The potential of ECS as a treatment for HD patients merits further consideration.
ACKNOWLEDGMENTS

I would like to express my sincerest gratitude to my advisor Dr. Sic L Chan. His valuable guidance, constant support and constructive criticism helped shape this dissertation. I would also like to acknowledge the support of my esteemed committee members Dr James Turkson, Dr Ella Bossy-Wetzel, and Dr Jack (Zixi) Cheng and also my program coordinator Dr Henry Daniell.

I would like to extend special thanks to all my lab members Dr Srinu Chigurupati, Dr Zelan Wei, Dr He Li, Weihong, Cherine, Adam, James, Spencer, Daniel for their continuous support.

I would thank Mrs Shobana Daniell, Dr Dolen Nameirakpam, Dr Jina Heisam, Dr Dheeraj Verma, Dr Meenu Verma, Dr Parineetha Bhat, Dr Veena, Dr Bhaskar, Dr Neeti, Dr Krishna Bhat, Dr Ana Pajor, Dr Rajendra Kedlaya for all the encouragement.

I am forever indebted to the love of my parents (Mr. Manohar Lal Baharani and Mrs. Laxmi Baharani), my sisters (Dr. Abhilasha Baharani and Ms. Aarzoo Baharani), my brother in law Vishel Venugopal and all the elders in my family with whose blessings, this goal in my life has been achieved.

My stay in the beautiful state of Florida has been delightful because of friends like Abhishek D, Abhishek V, Afsaan, Adam W, Aditya, Bethany, Bala, Bojanna, Gautam, Himanshu, Himansu, Jyoti, Kristi, Katie, Karan, Maggie, Mona, Monika Nisha, Niyatee, Prabhu, Pankaj A, Pankaj K, Simran, Udai, Vinit, William.

With the Grace of God; I hope to continue and succeed in the PhD program in Biomedical sciences at UCF.

Thank you all for being a part of my life.
CONTRIBUTIONS

I would like to thank Mohamed Mughal (NIA) and Srinivasuslu (Srinu) Chigurupati (NIA) for their contribution to the figures.
# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................................................ viii
LIST OF TABLES .................................................................................................................................................. ix
CHAPTER 1. INTRODUCTION ........................................................................................................................ 1
  1.1 Huntington’s Disease ................................................................................................................................. 1
  1.2 Anatomy .................................................................................................................................................. 3
  1.3 Genetics ................................................................................................................................................... 4
  1.4 Huntingtin Protein ................................................................................................................................... 5
  1.5 Transgenic mouse models of HD ........................................................................................................... 6
CHAPTER 2. LITERATURE REVIEW ............................................................................................................. 8
  2.1 Transcriptional dysregulation ................................................................................................................ 8
    2.1.1 Cyclic-AMP-response element-binding protein ........................................................................... 8
    2.1.2 Peroxisome proliferator-activated receptor-γ coactivator 1α ....................................................... 10
  2.2 Neurotrophins ......................................................................................................................................... 13
    2.2.1 Brain derived Neurotrophic factor ................................................................................................. 13
  2.3 Trk B ...................................................................................................................................................... 16
  2.4 The cell survival signaling pathway ...................................................................................................... 18
  2.5 Molecular chaperones in HD ................................................................................................................ 19
    2.5.1 Heat Shock Factor 1 ....................................................................................................................... 19
    2.5.2 Heat Shock Protein 70 / 40 ......................................................................................................... 20
  2.6 Depression and HD ................................................................................................................................ 23
  2.7 Electroconvulsive shock (ECS) ............................................................................................................ 24
CHAPTER 3. MATERIALS AND METHODS .............................................................................................. 28
  3.1 Mice and Electroconvulsive Shock Treatment ..................................................................................... 28
  3.2 Rotarod Test .......................................................................................................................................... 29
  3.3 Survival Study ......................................................................................................................................... 30
  3.4 Immunohistochemistry ......................................................................................................................... 30
  3.5 Quantification of Htt Aggregates and Damaged Neurons .................................................................... 31
  3.6 Immunoblotting ..................................................................................................................................... 31
  3.7 Statistical analysis .................................................................................................................................. 32
CHAPTER 4. RESULTS ...................................................................................................................................... 34
  4.1 ECS ameliorates striatal degeneration ................................................................................................. 34
  4.2 ECS reduces Htt protein aggregation .................................................................................................. 35
  4.3 ECS restores BDNF levels in the striatum of HD mice ......................................................................... 36
  4.4 ECS normalizes levels of heat shock proteins in the striatum of HD mice ........................................ 37
  4.5 ECS increases levels of PGC-1α ........................................................................................................... 37
CHAPTER 5. CONCLUSION .......................................................................................................................... 49
REFERENCES ...................................................................................................................................................... 53
LIST OF FIGURES

Figure 1 CAG repeats results in a polyglutamine tract which encodes Htt Protein ....................... 2
Figure 2 The Htt protein contains the HEAT repeat sequences ................................................... 6
Figure 3 Mutant and wild type Htt have been shown to be involved with a number of
transcription factors, one of them being Cyclic-AMP-response element-binding protein (CREB) binding protein (CBP) ............................................................................................... 9
Figure 4 PGC-1α forms a link between external physiological factors and mitochondrial
biogenesis. PGC-1α is activated by ROS, CREB and Akt. It also regulates the activities of CREB ..................................................................................................................................... 12
Figure 5 BDNF gene is a highly complex gene regulated by multiple promoters which lead to a
complex pattern of expression. ........................................................................................................ 17
Figure 6 Hsps regulate the cellular processes by modulating the misfolded protein and prevent
stalling of the normal physiologic function and thereby reducing the toxic effects of the
mutant protein. .................................................................................................................................. 22
Figure 7 ECS was delivered to anesthetized mice once a week via bilateral ear clip electrodes
using the Ugo Basile, ECT Unit 7801 ............................................................................................. 29
Figure 8 Electroconvulsive shock treatment ameliorates motor deficits and extends survival of
HD mice ............................................................................................................................................. 39
Figure 9 ECS treatment attenuates the degeneration of striatal neurons in HD mice. ................. 40
Figure 10 Images of cresyl violet stained coronal brain sections .................................................. 41
Figure 11 Images showing huntingtin (Htt) immunoreactive aggregates .................................... 42
Figure 12 ECS ameliorates deficits in levels of BDNF, and activated Akt and CREB, in the
striatum of HD mice ........................................................................................................................ 43
Figure 13 Levels of BDNF is elevated in striatal cells of HD mice treated with ECS ................... 44
Figure 14 Levels of pAkt elevated in striatal cells of HD mice treated with ECS. ......................... 45
Figure 15 ECS elevates levels of adaptive stress response proteins in the striatum of HD mice. 46
Figure 16 Levels of protein chaperones are elevated in striatal cells of HD mice treated with
ECS .................................................................................................................................................. 47
Figure 17 Levels of PGC-1α are reduced in the striatum of HD mice, and are partially or fully
restored by ECS treatment. .............................................................................................................. 48
LIST OF TABLES

Table 1 Primary Antibodies Used in the Study ................................................................. 32
CHAPTER 1. INTRODUCTION

1.1 Huntington’s Disease

American doctor George Huntington first described this neurological disorder in 1872. Huntington’s disease (HD) is an autosomal dominant progressive neurodegenerative disorder. It is caused by an abnormal expansion of the CAG repeat region in the exon 1 of HD gene (IT15). The CAG repeats result in a polyglutamine tract which encodes the huntingtin (Htt) protein (Fig 1). Htt is ubiquitously present in the body and the brain, but the accumulation of the mutant protein causes neuropathological changes therefore specifically affecting the brain (1).

The hallmark of HD is the degeneration of the striatal tissue (2,3). The gain of function mutant protein is toxic and causes impairment in the normal functioning of the Htt protein. The mutant Htt protein is cleaved which leads to the production of toxic polyglutamine tracts. The N-terminal polyglutamine is considered to be the cause of the toxicity by the mutant protein. The neuropathological damage in HD is expected to be due to the death of the striatal neurons. The precise mechanism underlying the degeneration of the striatal neurons in HD is still being investigated. Mutant Htt causes mitochondrial dysfunction which leads to calcium imbalances. The energetics and dynamics of the mitochondria are affected which lead to neuronal cell death (4). Impairment of the ubiquitin proteasome system and also axonal transport could be another cause of the neuronal loss / death. Transcriptional dysregulation has been widely considered as one of the causes of the striatal degeneration in HD. The transcriptional dysregulation of genes leads to the altered expression of various neuroprotective proteins in HD and is expected to play a key role in the specific loss of the tissue in HD (5,6,7,8).
HD is characterized by the *motor* and *cognitive* disturbances. The motor abnormalities are chorea, ataxia and uncoordinated movements. Chorea is expected to be caused due to neuronal dysfunction and motor impairment by the neuronal loss. The striatal pathology correlates with the severity of motor and cognitive dysfunction. This implies that the *striatal degeneration* is the *main cause of the disease process* but other structures such as the cortex and the hippocampus are also affected by mutant Htt (9). The complexity of the disease arises from the interplay between these regions and these interactions play an important role in the pathology of HD (2,6,10).
1.2 Anatomy

The cerebrum contains the *basal ganglia* which contains different nuclei. The basal ganglion is involved in the motor control. The basal ganglia comprises of *Striatum*, Globus pallidus, Subthalamic nuclei and Substantia nigra (*Fig 2A*). The basal ganglia circuitry involves a number of parallel loops which control and modulate the cortical outputs. Striatum is the major *receptive* component of the basal ganglia and it has three nuclei namely caudate, putamen and accumbens. Caudate nucleus of the striatum degenerates first followed by putamen and then nucleus accumbens in the later stages of the disease (12). Striatum is involved in regulating the motor activities. It also involved in the non motor behaviors such as cognition, attention and motivation (12).

The neurotransmitters present in the straitum are inhibitory, namely GABAergic (Gamma-aminobutyric acid). Almost 90% of the striatal neurons are *medium spiny neurons* which express GABA and about 10% are interneurons. The medium spiny neurons are further classified depending on the different neuropeptides. The two main classes are enkephalin-containing neurons and substance P containing neurons. Dopamine receptors are also widely expressed in the striatum. Since the striatum functions primarily in the regulation of movements and organization of motor activity it has a number of synaptic inputs from the cortex, midbrain and thalamus. Cortico-striatal neurons and thalamo-striatal inputs are excitatory since they are glutaminergic. Afferent from substantia nigra and ventral tegmental area are dopaminergic. The medium spiny neurons give rise to GABAergic efferents, which includes enkaphalinergic projections to lateral globus pallidus (11,12).
1.3 Genetics

HD is due to the defect in the exon 1 of the HD gene (*IT 15*) on the chromosome 4. The gene for HD was identified in 1993 by the HD collaborative research group (9,13). The abnormal expansion of CAG trinucleotide is genetically unstable. It is susceptible to expansion and occurs within the coding region of the gene. The gene is 17 codons downstream of the initiator codon ATG in exon 1, where CAG codes for glutamine.

Normally the HD gene contains repeats in the range of 17-20. The repeats between 27-35 are rare, and more than 36 may cause the disease. The higher the number of repeats the more an individual is prone to HD. Therefore the number of repeats is inversely related to the age of onset of the disease. The repeats between 27-35 are meiotically unstable and can expand (13), when transmitted through the paternal line, thus predisposing the individual to HD at an earlier age. The adult onset of the disease is due to 40-50 CAG repeats and the juvenile form of the disease is seen when the individual has 50 or more repeats. Incomplete penetrance is generally observed in individuals expressing 36-41 repeats. Homozygosity is associated with a more aggressive disease course (9,13,14).

Offspring of an HD parent have a 50% chance of inheriting the disease. The genetic abnormality equally affects both the genders and the repeats are seen in every generation. The CAG repeat length is a measure of the genetic testing for HD in the IT15 gene. An individual is classified as affected if the CAG repeat length is 39, termed as ‘pathologically fully penetrant’ (14,74).
1.4 Huntingtin Protein

The protein encoded by the IT15 gene is Htt which has the poly Q tract. It is a 350 kD protein which is expressed ubiquitously in the body. The function of normal Htt in neurons is unknown, although it may play a role in synaptic transmission. The Htt protein is about 3144 amino acid long and most of it is in the cytoplasm. Along with many other functions its being speculated that it is involved in transcriptional regulation. The pathogenic action of mutant Htt may result from gain of function (Fig 1) rather than loss of function because Htt deficient mice die early in embryonic development (8), while Htt +/- mice exhibit no or few neurological abnormalities (8,16). Htt is widely expressed in neuron throughout the brain and it remains unclear why striatal medium spiny neurons are particularly vulnerable to mutant Htt. However in vulnerable neurons mutant Htt forms intranuclear inclusions and a similar protein aggregation process occurs when mutant Htt is expressed in cultured cells and transgenic mice.

The Htt protein contains the HEAT (huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A and lipid kinase Tor) repeat sequences (Fig 4). There are 28-36 HEAT repeats in the Htt protein and each HEAT repeat comprises of approximately 50 degenerate amino acid sequences (16). These repeats have an increased helical content and form superhelical structures with hydrophobic cores. HEAT repeats are present in proteins involved in microtubule dynamics suggesting that these repeats play an important role in protein-protein interaction (13,93). Structural studies reveal the structure of Htt as an elongated superhelical solenoid with a diameter of ~200Å (15,16). The N-terminus of the protein contains a 17 amino acid sequence which is involved in the interaction of the protein with intracellular membrane bound organelles, such as the mitochondria, golgi apparatus and endoplasmic reticulum.
Transgenic mouse models of HD

Transgenic mouse models have become a necessity in studying various disease processes (17, 24). To study the neurological disease the mouse models should be robust and have limited variability. Along with a few other criteria they should be similar to the human disease process with minimum discrepancies and should display the neurobehavioral abnormalities observed in affected individuals (18, 20).

The mouse models being used to study HD fall into three broad categories. The first mouse models are the mice which express the fragments which include the first one / two exons. The second are the transgenic mice which express the full length human HD gene and the third are the knock in mice which have the pathogenic CAG repeats. The mouse models expressing the poly Q fragments of the mutant Htt are similar to human HD. (19, 20). Then mouse models show similarities too at the molecular and are neuropathologically similar to human HD. The ‘genetically accurate’ models do not exhibit the HD phenotype explicitly (20).

The first transgenic model developed was the R6/2 mouse model which contains the small N-terminal fragment of the exon 1 of the Htt protein. It has about 150 CAG repeats which give it a progressive phenotype. The model exhibits many of the behavioral and neuropathological
features of HD, but at the same time it has an excessive mutant Htt aggregate formation compared to human HD. On the contrary the neuronal loss is less compared to human HD and mice are resistant to excitotoxicity (20, 21).

The \textit{N-171-82Q} mouse model expresses a small N-terminal fragment (exon 1) of htt with \~82 CAG repeats under prion promoter. These mice show neuropathological similarities to the human HD and are extensively used to test various therapies for treating HD (20,21). These mice exhibit a robust phenotype, rapid disease onset and progression, well defined neurobehavioural abnormalities that can be quantified and neuropathological changes that accurately mirror human HD (151). Furthermore these mice exhibit a well-characterized progressive phenotype with moderate variability such that experimental groups can contain as few as 10 mice and provide the power to detect 10\% differences in many outcome measures.

Transgenic mice with full length Htt containing 48-89 CAG repeats have been produced. They show behavioral abnormalities like the human HD. A yeast artificial chromosome model (YAC128) has been shown to have cortico-striatal loss of neurons which lead to the behavioral abnormalities resembling human HD. The disadvantage of these mice is that there is variability among them and the time of phenotype development is delayed. Hence these mice are best suited for therapeutic trials (20). The transgenic mice developed with the Bacterial artificial chromosome (BAC) have 226 poly Q repeats and full length human huntingtin gene. The cortico–striatal neuronal loss is not specific as human HD (20, 21, 22).

Knock in mice are another set of genetically modified mice produced to test for HD pathogenesis. There have been 72-111 poly Q repeats been inserted in the mouse models and the behavioral and pathological phenotypes have been studied (22, 23, 24).
CHAPTER 2. LITERATURE REVIEW

2.1 Transcriptional dysregulation

2.1.1 Cyclic-AMP-response element-binding protein

Mutant Htt in HD samples have shown to form inclusions in the nuclei and neuritis of multiple neuronal populations (25,75). Full length mutant Htt or its fragments probably bind to the transcriptional factors which result in the regulation of gene transcription (30). Mutant and wild type Htt have been shown to be involved with a number of transcription factors, one of them being Cyclic-AMP-response element-binding protein (CREB) binding protein (CBP) (Fig 5A,B) (26,75).

CBP is present in the nucleus in normal cells, but CREB function is affected in a HD brain (31). There is interaction between mutant Htt and CBP, where the nuclear inclusions have been shown to interact with the transcriptional machinery thus causing toxicity. CBP has an acetyltransferase domain which acts as a coactivator for CREB-mediated transcription. The mutant Htt has been shown to bind with the acetyltransferase domain of CBP. This reduces the acetylation of histone (H3 and H4) by CBP. Histone deacetylase (HDAC) inhibitors can reverse this process, and improve the motor deficits in HD transgenic mouse models. The survival of HD-171-82Q mice is extended due to increase expression of protective proteins (13, 30, 75).
Figure 3 Mutant and wild type Htt have been shown to be involved with a number of transcription factors, one of them being Cyclic-AMP-response element-binding protein (CREB) binding protein (CBP).

A Wild type Htt have been shown to be involved with a number of transcription factors with CREB binding protein (CBP)

B Mutant Htt disrupts or its fragments probably bind to the transcriptional factors which result in the regulation of gene transcription.
2.1.2 Peroxisome proliferator-activated receptor-γ coactivator 1α

Mitochondrial dysfunction and oxidative damage has been considered to be the prime cause for neuronal loss. Reactive oxygen species (ROS) are generated primarily in the mitochondria during the various metabolic processes in the sub cellular organelles. The ROS are removed from the mitochondria by the free radical scavengers found on the inner mitochondrial membrane. The enzymatic removal of ROS occurs by Superoxide Dismutase (SOD). Once there is an imbalance created in the production of ROS and their clearance there is oxidative damage and that impacts the cell negatively. The cause of the neuronal loss due to oxidative damage is still being elucidated. Is it the toxicity that causes the mitochondrial defects or vice versa is still under debate (80,81).

PGC-1α (Peroxisome proliferator-activated receptor-γ coactivator 1α) is involved in energy homeostasis, adaptive thermogenesis, glucose metabolism and alpha oxidation of fatty acids. The tissues which have high energy needs, express increased levels of PGC-1α and PGC-1β, a close homolog (61,64). PGC-1α forms a link between external physiological factors and mitochondrial biogenesis. PGC-1α is activated by ROS, CREB and Akt and also regulates the activities of CREB (82).

Transcriptional coregulators are protein interacting with transcriptional factors. This activates or represses the function of transcribing genes. A coactivator activates, while co repressors repress the gene function. Htt interacts with many transcriptional factors which include CREB, and PGC-1α acts as a coactivator (62). PGC-1α deficient mice show HD like symptoms which are striatal degeneration and hyperkinetic movements (63). The striatal cell lines, transgenic HD mouse models and postmortem brain tissue from HD, all show a diminished PGC-1α function
Reduction in the levels of PGC-1α leads to improper energy metabolism and PGC-1α knockout mice show striatal degeneration. In HD there is an oxidative damage which affects the striatal neurons and PGC-1α protects the neurons against ROS. This indicates that energy metabolism, ROS defense and neurodegeneration appear to be controlled by PGC-1α. The knockout mice for PGC-1α show lesions in the brain and most predominantly in the striatum. These mice showed impaired thermoregulation and were hyperactive with constant limb clasping. This shows that PGC-1α plays a major role in the HD pathology because striatal degeneration is specific to HD and metabolic abnormalities are also observed in it. PGC-1α has been shown to be involved in oxidative stress. It reduces the level of ROS and also enhances ROS scavenging enzymes.

PGC-1α mRNA has also been shown to be reduced in HD striatal cell lines, HD knock in mouse models and HD patients. It has been shown that mutant Htt interferes with the transcription of the gene encoding PGC-1α. Since the expression of PGC-1α was reduced in the medium spiny neuron they were vulnerable to degeneration in HD. When a lentiviral vector expressing PGC-1α was used to overexpress it in the striatum then neuronal atrophy was prevented in the mouse model. Conversely PGC-1α is downregulated the mouse models exhibit behavioral neuropathological abnormalities. There has been a correlation between the transcriptional dysregulation and mitochondrial dysfunction in HD and PGC-1α is considered as a key player since it is inhibited by mutant Htt. Real time PCR data has also shown a decrease in the mitochondrial genes in human HD brain thus suggesting that PGC-1α could play a major role in the development or progression of HD.
Figure 4 PGC-1α forms a link between external physiological factors and mitochondrial biogenesis. PGC-1α is activated by ROS, CREB and Akt. It also regulates the activities of CREB.
2.2 Neurotrophins

Neurotrophins or Neurotrophic factors play a role in the growth, survival and differentiation of neurons. There is a reduction of these proteins in various neurological disorders; hence they seem to play a major role in the maintenance of the neurons. These proteins are secreted proteins and include a number of family members. Neurotrophins are transported after they have been processed by intracellular pathways. They are initially synthesized as pre / proneurotrophin precursors of about 27 kD, after which they are processed and mature neurotrophins of about 13kD are generated (32, 33).

They bind to two kinds of membrane receptors found on the plasma membrane. The receptors are namely; Tyrosine kinase receptors (Trk) and p75 pan-neurotrophin receptor (p75NTR). A number of Trk subtypes have been recognized which are classified according to their affinity to bind to various growth factors. Trk A binds to the Nerve growth factor (NGF) sand Trk B allows the binding of Brain derived neurotrophic factor (BDNF) and Neurotrophin (NT)-4/5. NT-3 binds preferentially to TrkC and with lower affinity to Trk B and Trk A (34, 35).

2.2.1 Brain derived Neurotrophic factor

BDNF was discovered in 1982 by Barde et al and his group. It is synthesized from a large precursor protein which is 32kD pro-BDNF protein. It moves to the intracellular compartments and undergoes proteolytic cleavage and appears in the extracellular compartments after maturation as a 14kD protein. Neurotrophins are generally released to the extracellular space without any triggers, but it is not the same for BDNF. Its secretion is activity dependent and
occurs on receiving depolarizing signals. BDNF can also be secreted as pro-BDNF which later undergoes cleavage by extracellular proteinases (76).

BDNF gene is a highly complex gene regulated by multiple promoters which lead to a complex pattern of expression. BDNF is synthesized as a ~32kD precursor protein called pro-BDNF. It forms the biologically active mature form of protein, after cleavage in the trans-golgi apparatus or the secretary granules.

Corticostriatal afferents convey the messages from the cortex to the striatum where it is integrated and processed. The corticostriatal synapse is the major site for the release of neurotransmitters and any defect at this convergence leads to improper functioning of the brain tissue. (76,77). BDNF is expressed in the entire CNS but its levels are higher in the hippocampus and the cerebral cortex. From here this neurotrophic factor is transported anterogradely to the striatum via the corticostriatal efferents.

Striatal neurons require BDNF for their health and survival; hence 95% of the striatal BDNF comes from the cortex. It has been shown by two groups that some of the BDNF in the striatum originates from the substantia nigra and becomes available to the striatal tissue (36). Cortical efferents are the primary source of BDNF, was first elucidated by Alter et al in 1997. BDNF has been shown to be the major regulator of synaptic plasticity. Its levels have been shown to increase during developmental stages. This shows that the factor is important for the normal functions of the neurons, its survival and differentiation. (36,77).

Some groups have co localized Htt and BDNF in the rat cerebral cortex and striatum. BDNF is involved in the survival of the striatal neurons that die in HD, therefore making it the molecular target for various therapeutic interventions (37, 41). It has been suggested that reduced levels of
BDNF play a major role in the onset or progression of HD. Many experimental analyses have shown that mutant Htt reduce transcriptional activity of BDNF promoters which leads to reduced transcription of the BDNF gene (Fig 6 A,B) (76). The mRNA levels of BDNF were found to be reduced in HD striatal samples versus the wild type striatal samples expressing mutant and wild type Htt respectively. The production of BDNF protein was more in wild type samples when compared to HD striatal samples. A knock in cell line supported the same results which were from heterozygous and homozygous mice with 109 CAG triplet repeats. These repeats were inserted into the endogenous mouse huntingtin gene (38, 39, 40).

Transcriptional dysregulation has been shown to be responsible for the reduction in BDNF levels in the cortex. The HD brain samples from patients have also revealed the reduction in BDNF in the cortex. This was further supported by studies in a huntingtin-knock out mouse, where the BDNF levels were shown to be reduced due to absence of wild type Htt protein. This shows that the stimulatory function of the wild type Htt is required for the appropriate BDNF levels (41,42).

BDNF is stimulated at the transcriptional level by different promoters which act independently. Wild type Htt is involved in the transcription process and the transcriptional control lies within the promoter II (Fig 5A). In the disease the mutant Htt interferes with the transcription of the BDNF gene through promoter II (Fig 5B). REST controls the activity of the repressor element 1 (RE1) within the promoter II. Htt attaches to the REST-interacting LIM domain protein (RILP) and prevents REST from binding to RE1. RILP is a cytoplasmic protein, therefore this allows REST to be recruited to the cytoplasm. If mutant Htt protein is present then REST is transported to the nucleus where it accumulates and binds to the RE1 sites. These RE1 sites are within the
BDNF exon II region of the gene, which results in the downregulation of the BDNF gene (40, 43, 44).

2.3 TrkB

BDNF binds to the tyrosine kinase receptor B (TrkB) and leads to the activation of the intracellular signaling pathway. The biological activity of BDNF is accomplished when it binds to its receptor. TrkB increases in the striatal and cortical neurons after excitotoxic damage and mutant Htt reduces the TrkB levels in HD (78). This reduced TrkB level corresponds to the mRNA expression in the HD mice. (39, 46, 78).

It has been shown that TrkB levels decrease due to mutant Htt and this reduction was associated with the decrease in CRE-TrkB promoter activity. This could be inferred as reduced CREB mediated transcription might lead to lower expression of TrkB expression in HD cell lines. Therefore in neurodegenerative disease where TrkB levels are decreased it will affect the BDNF – TrkB interaction which eventually affects the prosurvival signaling pathways (40, 45, 47, 78).
Figure 5 BDNF gene is a highly complex gene regulated by multiple promoters which lead to a complex pattern of expression.

A Wild type Htt is involved in the transcription process and the transcriptional control lies within the promoter II

B Mutant Htt interferes with the transcription of the BDNF gene through promoter II
2.4 The cell survival signaling pathway

Akt (also known as Protein Kinase B; PKB) is a potent pro-survival kinase which is involved in a number of cellular processes, including mitogenesis, cell size and survival. This serine threonine kinase is involved in the cell survival signaling pathway and this is mediated by phosphorylating other substrates and enhancing the survival effects in the neurons (48). The pathway is affected in neurodegenerative disorders including HD. Htt is phosphorylated on Ser-421 (S421) by Akt and this protects it against the toxicity of the mutant Htt with the poly Q in vitro (49).

Phospho Akt is found in the nuclear inclusion of the HD mice while Akt localizes it the cytoplasm (48). Phospho Akt is downregulated in rat striatum of N171-82Q HD rat models. Postmortem brain samples of HD patients have also shown a decrease in the activated form of Akt (48). If there is a decrease in the levels of Akt / Phospho Akt then the survival pathway is not activated which leads to the increased toxicity of the mutant Htt in the cell (50).

Apart from the activation of the survival pathway, Akt is involved in the variety of other roles in the central nervous system, which includes axon elongation, branching of sensory neurons, modulation of synaptic plasticity and many others. Since Akt is involved in many other pathways; that require transcriptional regulation, cell proliferation and apoptosis; it plays an important role in the neurodegenerative disorders (50, 51).
2.5 Molecular chaperones in HD

2.5.1 Heat Shock Factor 1

Heat Shock Factor 1 (HSF 1) is a transcription factor which binds to the heat shock elements (HSE) of Hsp genes and causes induction of Hsp proteins under stress conditions (Fig 7). Under stress conditions HSF 1 is dissociated from Hsp’s and forms a trimer before its translocation into the nucleus. HSF1 is phosphorylated at multiple sites and binds to the heat shock elements (HSE) on the promoter region of the target genes (52,79). HSF1 is a master regulator of cell survival under stress conditions. The function of HSF1 is regulated by may unknown genes among which the known are the Hsp family of genes. HSF 1 also regulates growth factors genes which makes it the regulator of various physiologic processes. The tissues containing polyglutamine expansions, when over expressed with HSF1 show an attenuation of the disease process (52, 54). The expression of HSF 1 in non neural tissues also leads to an increase in the survival of HD mice. Chromatin immunoprecipitation has shown that HSF1 binds to a large number of human genes in vivo. The binding takes place even under normal conditions, the gene need not be induced by stress to bind (52).
Heat Shock Proteins (Hsp) are molecular chaperones that play a major role in protein homeostasis (*proteostasis*) (*Fig 8*). Proteostasis is a process which ensures that the cells express properly folded proteins. The protein homeostasis machinery comes into play and the proteins are properly expressed, folded and removed to prevent the cells from the toxic effects of misfolded proteins. Molecular chaperones help in proteostasis and prevent the accumulation of the misfolded proteins. They are highly conserved and ubiquitously expressed proteins. They are found in all subcellular compartments under normal and stressed conditions. The expression of molecular chaperones is regulated by various stresses. Stress induced molecular chaperones are called Heat shock proteins (Hsps) and their gene families are classified according to their molecular mass as Hsp 100, Hsp90, Hsp 70, Hsp 60, Hsp 40 and small Hsps (sHsps). Cells show an increase in the heat shock response under stress conditions and survive. If the cells are unable to induce enough heat shock proteins they succumb to stress and die. There is cascade of events start with the activation of the heat shock proteins by HSF1 (52,79).
There are various types of stresses which can affect a cell. They are i) environmental stress such as temperature change ii) chemical stress such as ROS iii) pathophysiological states causing stress such as bacterial, viral infection etc. The cell expresses Hsps, genes encoding chaperones and other proteins to reduce the effect of stress and maintain the proteasomal homeostasis. Proteasomes are very large complex protein complexes involved in the regulation of misfolded proteins. The lysis of these proteasomes is carried out by proteases, termed proteolysis. In diseased conditions like HD either the proteosomal machinery is exhausted in clearing up or the aggregation of the protein escapes the misfolded protein degradation machinery and toxic protein affects the cellular functions (55).

The poly Q expansion in HD acts as a stress factor which leads to the protective stress response in the form of upregulation of Hsps. (53). Hsps have been shown to reduce poly Q aggregation (55). Since HSPs are molecular chaperones and they protect the cells by preventing misfolding and / or aggregation they protect against neurodegeneration caused by poly Q expansion. Therefore Hsps help in maintaining normal protein structure and function (79).

Hsps are highly conserved between different species. Most of these proteins donot act alone but form complexes consisting of an Hsp and a co-chaperone. Co-chaperones are proteins that modulate the activity of primary chaperones (57). In humans there are about eleven genes encoding Hsp 70, all having a conserved N-terminal ATPase domain and a C-terminal substrate binding domain. Hsp 40 binds to Hsp 70 and promotes ATP hydrolysis and acts as a conformational switch to allow its interaction with nonnative substrates (58) (Fig 6).
Toxicity in HD results due to the presence of mutant Htt. The misfolded proteins accumulate in the cytoplasm as aggregates which might disrupt the ubiquitin-proteasome system (UPS). If the UPS is disrupted there is increase in the misfolded proteins in the cytoplasm (52,59). In HD mouse models endogenous Hsp 70 has shown to alleviate the symptoms of the disease, which has been confirmed by behavioral tests (52,57). In these mouse models the presence of Hsp 70 acts as a protective mechanism, protecting the cell to the toxic effects of mutant Htt. Hsp 70 has been shown to regulate the toxicity of the mutant Htt in the mouse model of HD. Hsp 70 and Hsp 40 interact with Htt by stabilizing the mutant protein. They suppress the accumulation of annular and spherical oligomeric forms of mutant Htt. Based on this; the primary function of Hsp 70 in HD is to prevent the formation of toxic monomers and oligomers. It is assumed that Hsp’s also prevent the interaction of mutant Htt with the cellular components which are involved in the physiological processes in a cell (58, 60).

Figure 6 Hsps regulate the cellular processes by modulating the misfolded protein and prevent stalling of the normal physiologic function and thereby reducing the toxic effects of the mutant protein.
2.6 Depression and HD

Depression is the most common psychiatric symptom that is observed in HD patients. Patients suffering from depression need symptomatic treatment which is mostly in the form of drugs which have a number of harmful side effects. Patients with HD are more likely to commit suicide compared to the population suffering from a mental disorder (110). Suicidal tendency was highest among the gene carriers who were at the threshold of being diagnosed with HD (113). The suicidal thoughts have been linked to the patients losing their ability to function independently as well as to depression leading to suicidal thoughts. The patients need special attention and a check needs to be performed to see if they are developing suicidal tendencies. A number of neurological problems involving the patient undergoing chronic depression which remains untreated with drugs led to the introduction of ECS for the treatment of drug resistant depression (74,85). Various neurodegenerative diseases have been correlated with dementia and increased suicide rates. There seems to be an association between dementia and suicide. There are functional impairments which lead to the inability to think and plan which leads to self destructive acts (87, 88). Suicidal tendencies have been noticed in patients suffering with Alzheimers disease (92). Dementia is also seen in about 60% of the patients with Parkinson disease (93).

HD patients show neurological symptoms before they show any signs of dementia. The attempt to suicide and suicidal deaths were four times the normal general population in HD patients. The deaths have been shown to occur at an early stage of the disease and hence if treated early could reduce the suffering of the patients as well as improve the lifestyle of the individual. Since HD is a genetic disorder it can be detected at an early stage if an individual is predisposed to it. A
treatment to alleviate the symptoms of dementia and depression could be beneficial to improve the quality of life of the patients suffering. (92, 93, 94, 95).

2.7 Electroconvulsive shock (ECS)

Electroconvulsive shock (ECS) is defined as a medical procedure in which a brief electrical stimulus of variable frequency applied to the scalp via electrodes is used to induce a cerebral seizure (134). Historically, ECS has been used mainly to treat depressive disorders since 1938 (134,135). Development of key psychotropic drugs one after another in the mid-20th century opened a revolutionary era of modern psychopharmacology, which made ECS relatively unpopular. However the presence of drug-resistant depression has necessitated the revival of ECS. ECS has become indispensable choice for the severest form of depression than any other forms of treatment (136). A recent review that pools data from 26 studies that result in an increase in global improvement for people with schizophrenia (137). The antidepressant effect is reliable and can persist more than a year (137). ECS has improved the quality of life for nearly 80% of patients. Suicide attempts are relatively rare after ECS.

The science of ECS has progressed rapidly over the last 20 years (134,138, 139,140,141,142). ECS uses precisely calculated electrical currents administered in a controlled setting to achieve the most benefit with the fewest possible risks (134,141,142). Numerous studies have demonstrated the safety and efficacy of ECS for various psychiatric conditions, and it has been approved by the Food and Drug Administration for the treatment of refractory depression. The American Psychiatric task force identifies no absolute contradictions and approves ECS as “safe
and effective” treatment for psychiatric disorders including depressive illness, schizophrenia, catonia and mania (135). Responsiveness to ECS does not abate with age which is a major risk factor in many neurological disorders. Despite an increasing body of evidence that ECS is effective, safe and has no detrimental effects, its dissemination appears still limited (136,138,139,141,143). One reason may be its public misconceptions, knowledge, and inaccurate depictions (144,145). Another reason may be that its neurobiological mechanisms are far from elucidated. It is hoped that once the biological benefits of ECS is understood in depth, a less invasive alternative, or a pharmaceutical or gene-technological replacement might be developed. Apart from its use in psychiatry ECS has been reported to have therapeutic benefits for the treatment of certain neurological conditions independent of its psychiatric effect (146). These include Parkinson’s disease, neuroleptic malignation syndrome, and aggressive behavioral disorders following brain injury, certain forms of epilepsy, and some forms of delirium due to toxic metabolic encephalopathies (134).

ECS was initially given in a sinusoidal waveform, which is less efficient. The electrical impulse provided is below the depolarization threshold of the neurons and hence produces no significant changes in the patient. The *brief pulse stimulus* was introduced later which allows the delivery of full current amplitude instantaneously thus making the procedure efficient. ECS was initially delivered bilaterally which later was changed to the unilateral way. Since both the ways did not show significant difference the bilateral electrode placement was restored.

Neuroplasticity has an important role to play in the recovery from depression (74). Antidepressant drugs and electroconvulsive shock has been shown to elevate the levels of neurotrophins. This includes BDNF and its receptor Trk B, which increase the neurogenesis and
the number of synapses in the different brain regions. This indicates neuronal plasticity may be able to alleviate the depression and BDNF can play a major role in the rewiring of the dysfunctional neuronal network. It has been shown that ECS is the most efficient and safe treatment against depression. It leads to an increase in the BDNF mRNA within minutes to hours of treatment (74,84). BDNF is responsible for the growth and survival of the neurons and has been shown to increase after ECS treatment (84, 86). ECS induces a number of complex brain events which can be broadly classified as intracellular signal transduction and regulation of gene expression. Intracellular signal transduction is a process which converts a chemical / mechanical stimulus to the receptor into a specific cellular response. This response creates a change in the cell within the cytoplasmic compartment or the nucleus. The regulation of the information contained in the genes to convert them into gene products is termed as regulation of gene expression. This gene expression is modulated by transcription or by post-translational modification of a protein. These signaling mechanisms and regulation of the gene expression by ECS produces a significant change in the condition of patients suffering from depressive disorders; as mentioned above; by an upregulation of neurotrophic factors such as BDNF (84,89,90).

ECS can lead to rapid and significant improvements in symptoms compared to psychotherapy or medication, it should be considered as a treatment in the management of depression in patients with HD, eventually contributing to decrement of the number of depression-driven suicides (147,148,149,150). The America Psychiatric Association recommends that ECS could be delivered to patients who respond to the treatment and are drug resistant otherwise (87,91) The reduction in depression is correlated with an increase in BDNF, which has been shown to be
neuroprotective. Thus ECS could be *neurorestorative* in HD patients along with alleviating depression. Hence it could be an efficient *supportive therapy* in improving the conditions of HD patients and attenuate the disease condition.
CHAPTER 3. MATERIALS AND METHODS

3.1 Mice and Electroconvulsive Shock Treatment

HD-N171-82Q mice were purchased from the Jackson Laboratories (Bar Harbor, ME) and were maintained on the B6C3F1 background; offspring were identified by PCR analysis using genomic DNA extracted from tail biopsies to distinguish transgene-bearing mice from their wild type littermates (95). Mice were maintained under usual laboratory conditions that included ad libitum feeding and drinking, in a non enriched environment (96). Experiments were performed on 2 month old male mice that were assigned randomly to control and ECS treatment groups. All procedures were approved by the National Institute on Aging Animal Care and Use Committee. ECS was delivered to anesthetized mice once a week via bilateral ear clip electrodes using the Ugo Basile, ECT Unit 7801. The stimulus current was 50 mA and the stimulus duration was 0.2s. The presence of seizures immediately after the shock was confirmed by observing the extension of all limbs and forward head extension that normally last for 10-15 s. The sham groups were handled identically to the ECS-treated animals except no current was applied. ECS- and sham-treated mice were returned to their cages 10 min afterwards (Fig 7)
Figure 7 ECS was delivered to anesthetized mice once a week via bilateral ear clip electrodes using the Ugo Basile, ECT Unit 7801.

3.2 Rotarod Test

Motor coordination and balance were evaluated using an accelerating rotary rod apparatus (Columbus Instrument, OH) as described previously (97). Mice were trained to use the rotarod apparatus during a 2 min trial (4 rpm) on the day before the first day of testing. On test days, mice were placed on the rotarod for three trials for a maximal 4 min at accelerating speeds from 440 rpm and maintenance at 40 rpm after 4 min. Each trial was separated by a 30 min rest period to alleviate stress and fatigue. The rotarod tests were conducted weekly to follow the progression
of the disease process; latency to fall and falling times for each mouse were recorded by a trained observer blind to the treatment group of the mice.

3.3 Survival Study

Once motor symptoms appeared the HD mice were examined twice daily in the early morning and late afternoon. Mice unable to right themselves after being placed on their backs and unable to initiate movement after being gently prodded for 30s were euthanized; their age at this point was considered the age of death. Deaths that occurred overnight were recorded the next morning.

3.4 Immunohistochemistry

Immediately after being anesthetized with an overdose of isoflurane, mice were perfused transcardially with saline, followed by 4% paraformaldehyde (PFA) in PBS (pH 7.4). Brains were post-fixed in 4% PFA for 24 h, and then cryoprotected in 30% sucrose/PBS for 48 h. Serial coronal sections (10 µm thickness) cut through the entire striatum with a freezing microtome (Microm HM 505 N) were collected on slides. Sections were blocked with 5% normal serum in 0.1% Triton X-100 in PBS for 30 min at room temperature followed by incubation overnight at 4°C with primary antibodies against N-terminal Htt (EM48, Chemicon; 1:400 dilution) and NeuN (Chemicon; 1:400 dilution). Tissue sections were incubated with Alexa568- or Alexa488-conjugated secondary antibodies (1:200, Molecular Probes) appropriate for the specific primary antibodies. The sections were then washed with 0.05% Tween-20 in phosphate-buffered saline
for 1h and counterstained with 4′6′-diamidino-2-phenylindole (DAPI) (50ng/ml) for 30s, washed, and mounted in FluorSave medium (Calbiochem). Additional sections were stained with Fluoro Jade C (98).

3.5 Quantification of Htt Aggregates and Damaged Neurons

Images of EM48-positive neuronal inclusions and Fluoro Jade C-positive neurons were obtained by scanning 1012 coronal sections spread over the anterior - posterior extent of the striatum (inter-section distance: 400 m), using a 20 objective on a Nikon 80i Research Upright Microscope equipped with image acquisition software (Qimaging Retiga 2000). All images were segmented using the same light threshold, mask smoothing and object size filters. Total area of pixel intensity was measured with the automated measurement tools in IP lab software (BD Biosciences- Bio-imaging). The total density was averaged and expressed as normalized, corrected OD. All brain specimens were coded and analyses were performed by an investigator blinded as to the genotype and treatment group of the mice.

3.6 Immunoblotting

Protein quantification, electrophoretic separation and transfer to nitrocellulose membranes were performed. Membranes were incubated in blocking solution (5% milk in Tween Tris Buffered Saline; TTBS) overnight at 4°C followed by 1 h incubation in primary antibody diluted in blocking solution at room temperature. Membranes were then incubated for 1 h in
secondary antibody conjugated to horseradish peroxidase (Vector Laboratories) and bands were visualized using a chemiluminiscence detection kit (ECL, Amersham). Membranes were stripped and re-probed with the actin antibody to verify and normalize protein loading (50 µg total protein, unless stated otherwise). For immunodetection of blots, enhanced chemiluminescence (ECL, Amersham) was applied. Immunoreactive bands were quantified using NIH Image software. Information on the primary antibodies used in this study, including the source, dilution and molecular weight of the antigen can be found in Table 1.

**Table 1 Primary Antibodies Used in the Study**

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Secondary Antibody</th>
<th>Dilution</th>
<th>Company</th>
<th>Mol wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREB</td>
<td>Rabbit mAb</td>
<td>1:1000</td>
<td>Cell Signaling</td>
<td>43 kD</td>
</tr>
<tr>
<td>pCREB</td>
<td>Rabbit mAb</td>
<td>1:1000</td>
<td>Cell Signaling</td>
<td>43 kD</td>
</tr>
<tr>
<td>Akt</td>
<td>Rabbit pAb</td>
<td>1:800</td>
<td>Cell Signaling</td>
<td>60kD</td>
</tr>
<tr>
<td>pAkt</td>
<td>Rabbit mAb</td>
<td>1:800</td>
<td>Cell Signaling</td>
<td>60kD</td>
</tr>
<tr>
<td>Hsp 70</td>
<td>Mouse mAb</td>
<td>1:1500</td>
<td>Abcam</td>
<td>70-78kD</td>
</tr>
<tr>
<td>Hsp 40</td>
<td>Rabbit pAb</td>
<td>1:1500</td>
<td>Abcam</td>
<td>40kD</td>
</tr>
<tr>
<td>Hsf1</td>
<td>Rabbit pAb</td>
<td>1:500</td>
<td>Abcam</td>
<td>83kD</td>
</tr>
<tr>
<td>BDNF</td>
<td>Rabbit pAb</td>
<td>1:1000</td>
<td>Santa Cruz</td>
<td>&lt;20kD</td>
</tr>
<tr>
<td>PGC1∞</td>
<td>Rabbit pAb</td>
<td>1:5000</td>
<td>Santa Cruz</td>
<td>85-90kD</td>
</tr>
</tbody>
</table>

3.7 Statistical analysis

Kaplan-Meier survival data was analyzed using log-rank test for trend. All other data were analyzed using one-way ANOVA with Dunnett’s post-hoc test for pairwise comparisons or Students t-test as appropriate. These statistical analyses were performed using GraphPad Prism
version 5.00 for Windows (GraphPad Software, San Diego, CA). p values < 0.05 were considered significant.
CHAPTER 4. RESULTS

Electroconvulsive shock ameliorates motor deficits and improves survival of HD-N171-82Q mice. Previous studies showed that N171-82Q HD mice have an abbreviated life span of about 21-22 weeks and exhibit motor deficits beginning at 14-16 weeks of age (95). To determine whether ECS provides significant clinical benefits in an animal model, we assessed the impact of ECS on the development of behavioral symptoms, and on the survival, of male HD-N171-82Q HD mice. Beginning at 2 months of age, 20 wild-type and 20 HD mice were randomly assigned to either an ECS treatment group or a sham control group (10 HD and 10 wild-type mice per group); ECS was administered once weekly. HD mice in the ECS group lived significantly longer, by an average of 2 weeks, than did HD mice in the sham control group (Fig. 13A). No wild-type mice in either ECS or sham groups died during the 200 day course of this study. ECS also prevented the progressive weight loss that occurred in sham-treated control HD mice (Fig. 13 B). To determine the impact of ECS on motor function, we subjected 14 week-old mice to rotarod testing. We found that latency (time until falling) was significantly greater and the frequency of falling was significantly less in ECS-treated compared to sham-treated HD mice (Fig. 13C,D). Collectively, these data demonstrate that ECS treatment ameliorates the behavioral deficits and extends the survival of HD mice.

4.1 ECS ameliorates striatal degeneration

To determine whether improved motor function and improved survival of HD mice treated with ECS results from a slowed progression of the neurodegenerative process, we performed histological analyses to evaluate neuronal degeneration in the striatum in brain tissue sections.
from ECS- and sham-treated HD and wild-type mice that were killed at 16 weeks of age (an early symptomatic time point). In HD-N171-82Q mice, neuronal loss occurs primarily in the striatum and cerebral cortex resulting in a corresponding enlargement of the lateral ventricles (95,96). Degenerating neurons in HD-N171-82Q mice are found predominantly in both cortex and striatum beginning at approximately 14 weeks of age (95), and so we evaluated neuronal degeneration at 16 weeks of age when neurodegeneration is prominent. The number of Fluoro Jade C-labeled degenerating striatal cells was significantly lower in ECS-treated HD mice compared to sham-treated control HD mice (Fig. 14A). The number of NeuN-labeled neurons was significantly greater in the striatum of ECS-treated HD mice compared to control HD mice (Fig. 14B). Progressive striatal and cortical atrophy results in bilateral ventricular enlargement and flattening of the medial aspect of the striatum, accompanied by thinning of the cerebral cortex in HD mice (10). As expected, the lateral ventricle size in 16 week-old sham control HD mice was significantly greater than that of age-matched wild type mice (Fig. 14C). In contrast, the ventricles of 16 week-old ECS treated HD mice were significantly smaller than the ventricles of control HD mice, and similar in size to wild-type mice (Fig. 14C). These findings suggest that ECS protects striatal and cortical neurons against the neurodegenerative effects of mutant Htt.

4.2 ECS reduces Htt protein aggregation

Mutant Htt forms abnormal intracellular aggregates in degenerating neurons in the striatum and cortex of HD mice (96-99), and Htt aggregates are associated with neuronal dysfunction and death (100). We therefore determined whether ECS reduced neuronal degeneration by
suppressing Htt aggregate formation or by protecting neurons downstream of Htt aggregate formation. The density of Htt aggregates in the striatum, as measured by image analysis of brain sections immunostained with an Htt antibody, was significantly lower in ECS treated HD mice compared to sham control HD mice (Fig. 14D). No Htt immunoreactivity was detected in brain sections from wild-type mice.

4.3 ECS restores BDNF levels in the striatum of HD mice

The transcription of the bdnf gene has been reported to be impeded by mutant Htt protein, and BDNF protein levels are reduced in the striatum of HD patients and Htt mutant mice (96,99,100,101). ECS can increase BDNF levels acutely (within minutes to a few hours) in the hippocampus and cerebral cortex of normal mice, and this effect of ECS is potentiated by prior ECS treatments (102). We therefore determined the effects of ECS on BDNF levels in the striatum of HD mice. For these and subsequent biochemical and immunohistochemical analyses the mice were given a final ECS or sham treatment at 13 weeks of age, and were sacrificed 1 week later. BDNF protein levels, measured by both immunoblot (Fig. 17A) and ELISA (Fig. 17B) analyses, were significantly lower in the striatum of HD mice compared to wild-type mice. BDNF levels were significantly greater in ECS-treated HD mice compared to sham-treated HD mice (Fig. 17A, B). We next measured striatal levels of activated (phosphorylated) Akt and CREB, a kinase and transcription factor involved in BDNF signaling and BDNF transcription induction, respectively. The levels of p-Akt and p-CREB were significantly lower in the striatum of sham-treated HD mice compared to wild-type mice (Fig. 19). In contrast, levels of p-Akt and
p-CREB were similar in the striatum of ECS-treated HD mice and wild-type mice (Fig. 19), suggesting that ECS can prevent the impairment of Akt- and CREB mediated signaling caused by mutant Htt.

4.4 ECS normalizes levels of heat shock proteins in the striatum of HD mice

Heat-shock proteins (Hsps) are protein chaperones that prevent misfolding and aggregation of newly synthesized mutant proteins and damaged normal proteins. Recent findings suggest that levels of several Hsps decrease in striatal neurons in HD, possibly as the result of sequestration of mutant Htt (103,104). Additional findings suggest that mutant Htt can sequester heat-shock factor 1 (HSF1), a transcription factor responsible for the induction of many Hsps (105). To determine whether reduced aggregation may be attributed to increased expression of Hsps, we performed immunoblotting of striatal and cortical homogenates from HD-N171-82Q and wild-type mice treated with ECS or sham. ECS treatment markedly increased the level of Hsp70 and Hsp40 protein in HD mice (Fig. 20A). The increase of Hsps was associated with an increased in HSF1 protein (Fig. 20B). These results suggest that ECS increases expression of Hsps in HD mice via a mechanism that may be dependent on HSF1.

4.5 ECS increases levels of PGC-1α

Previous findings suggest that mitochondrial dysfunction plays a role in the pathogenesis of HD, possibly as a result of interactions of mutant Htt with the outer mitochondrial membrane leading
to perturbed mitochondrial calcium handling and impaired energy production (106,107). Emerging findings suggest that the transcriptional co-activator PGC1-α plays major roles in regulating mitochondrial biogenesis, function and vulnerability to stress (108). Consistent with a previous report (109), we found that levels of PGC1-α were significantly lower in the striatum of HD mice compared to wild-type mice (Fig. 22). ECS-treatment resulted in a significant preservation of PGC1-α levels in HD mice. There was a trend towards increased PGC1-α levels in ECS-treated wild-type mice (Fig. 22). Collectively, these findings suggest that that ECS may up-regulate multiple adaptive cellular stress response proteins to protect striatal neurons against pathogenic actions of mutant Htt.
Figure 8 Electroconvulsive shock treatment ameliorates motor deficits and extends survival of HD mice.

A. HD-N171-82Q HD mice were treated once weekly with either ECS (n = 10) or sham control (n = 10) beginning at 2 months of age and deaths were recorded. The Kaplan-Meier analysis revealed a significantly greater survival of mice in the ECS group (p<0.05). B. Body weights of wild-type (WT) and HD mice in the ECS or sham control (CTL) groups. C and D. Results of rotarod analysis of motor function (14 week-old mice) showing falling latency (C) and number of falls (D) for WT and HD mice in the CTL and ECS treatment groups. Values are the mean and SEM (n = 10 mice per group). *p < 0.05, **p<0.01.
Figure 9 ECS treatment attenuates the degeneration of striatal neurons in HD mice.

A. Images of FluorJade C (FJ-C)-stained cells (upper micrographs; scalebar = 250 µm) and results of counts of FJ-C stained cells (lower graph) in 16 week-old WT and HD mice in ECS or sham control treatment groups. B. Images of NeuN-stained cells (upper micrographs; scale bar = 250 µm) and results of counts of NeuN-positive cells (lower graph) in 16 week-old WT and HD mice in ECS or sham control treatment groups.
Figure 10 Images of cresyl violet stained coronal brain sections

Images of cresyl violet stained coronal brain sections (upper micrographs) and results of measurements of the area of the lateral ventricle (lower graph) in 16 week-old WT and HD mice in ECS or sham control treatment groups. Scale bar = 250 µm.
Figure 11 Images showing huntingtin (Htt) immunoreactive aggregates

Images showing huntingtin (Htt) immunoreactive aggregates (red; upper micrographs; scale bar = 250 µm) and results of densitometric analysis of Htt immunoreactivity (lower graph) in 16 week-old HD mice in ECS or sham control treatment groups. Values are the mean and SEM (n = 9-10 mice per group). *p<0.05, **p<0.01, ***p<0.001
Figure 12 ECS ameliorates deficits in levels of BDNF, and activated Akt and CREB, in the striatum of HD mice.

A. A representative immunoblot (left) and results of densitometric analysis of BDNF protein levels in striatal tissue samples from ECS-treated and control (C) HD and WT mice. B. BDNF protein levels, measured by ELISA analysis, in samples of striatal tissue from ECS treated and control HD and WT mice. C. A representative immunoblot (upper) and results of densitometric analysis of phospho-Akt protein levels in striatal tissue samples from ECS-treated and control (C) HD and WT mice. D. A representative immunoblot (upper) and results of densitometric analysis of phospho-CREB protein levels in striatal tissue samples from ECS-treated and control (C) HD and WT mice. Values are the mean and SEM (n = 8-10 mice per group). *p<0.05, **p<0.01, ***p<0.001
Figure 13 Levels of BDNF is elevated in striatal cells of HD mice treated with ECS.

Levels of BDNF are elevated in striatal cells of HD mice treated with ECS. Images showing BDNF (red in micrographs at right) and results of densitometric analysis of immunoreactivities (graphs) in 16 week-old HD mice in ECS or sham control treatment groups. Values are the mean and SEM (n = 10 mice per group). **p<0.01, ***p<0.001. Scale bar = 20 micrometers.
Figure 14 Levels of pAkt elevated in striatal cells of HD mice treated with ECS.

Levels of pAkt elevated in striatal cells of HD mice treated with ECS. Images showing pAkt (red in micrographs at right) and results of densitometric analysis of immunoreactivities (graphs) in 16 week-old HD mice in ECS or sham control treatment groups. Values are the mean and SEM (n = 10 mice per group). **p<0.01, ***p<0.001. Scale bar = 20 micrometers.
Figure 15 ECS elevates levels of adaptive stress response proteins in the striatum of HD mice.

ECS elevates levels of adaptive stress response proteins in the striatum of HD mice. A representative immunoblot (left) and results of densitometric analysis of HSP 70 and HSP 40 protein levels in striatal tissue samples from ECS-treated and control (C) HD and WT mice. B. An immunoblot (left) and results of densitometric analysis of HSF-1 protein levels in striatal tissue samples from the indicated groups of mice. Values are the mean and SEM (n = 8-10 mice per group). *p<0.05.
Figure 16 Levels of protein chaperones are elevated in striatal cells of HD mice treated with ECS.

Levels of protein chaperones are elevated in striatal cells of HD mice treated with ECS. Images showing HSP70 and HSP40 (red in micrographs at right) and results of densitometric analysis of immunoreactivities (graphs) in 16 week-old HD mice in ECS or sham control treatment groups. Values are the mean and SEM (n = 10 mice per group). **p<0.01, ***p<0.001. Scale bar = 20 micrometers.
Figure 17 Levels of PGC-1α are reduced in the striatum of HD mice, and are partially or fully restored by ECS treatment.

Levels of PGC-1α are reduced in the striatum of HD mice, and are partially or fully restored by ECS treatment. Immunoblots (upper) and results of densitometric analysis (lower) of protein levels of PGC-1α (C) in striatal tissue samples from the indicated groups of mice. Values are the mean and SEM (n = 8-10 mice per group). *p<0.05, **p<0.01, ***p<0.001.
CHAPTER 5. CONCLUSION

Once-weekly ECS treatments delayed disease onset and extended survival in Htt mutant mice. Biochemical and histological analyses demonstrated a reduction in neuronal degeneration, and elevated levels of neuroprotective proteins (BDNF, protein chaperones and PGC1α) in the striatum of ECS-treated HD mice compared to sham-treated HD mice. These results in an animal model of HD suggest that ECS can counteract the pathogenic actions of mutant Htt, thereby preserving the viability and function of striatal neurons. There have been several reports describing beneficial effects of ECS in relieving depression in HD patients (110,111,112), and in some cases motor symptoms also abated (113). However, there have been no controlled studies of ECS treatment in symptomatic HD patients, nor any attempts to delay the onset of HD with periodic ECS treatments.

BDNF plays critical roles in synaptic plasticity and neuronal survival in many brain regions including those affected in HD. Previous studies have shown that endogenous BDNF (117) and BDNF delivered directly or by transgenesis (114-116), can protect striatal and cortical neurons in experimental models of HD. We found that striatal BDNF levels were reduced in HD compared to wild-type mice, and that BDNF levels were significantly increased in HD mice that had been maintained on long-term ECS treatment compared to sham control HD mice. The elevation of BDNF levels may mediate, at least in part, the retardation of disease onset and extension of survival by ECS in the HD mice. Consistent with the latter possibility, it was reported that paroxetine and seratraline, two other anti-depressant treatments that increase BDNF levels in the striatum and cortex, also delay disease onset and extend survival in HD mice (118-119). BDNF may protect neurons against excitotoxic, metabolic and oxidative stress believed to be involved
in the death of neurons in HD. Indeed, BDNF can protect neurons against glutamate receptor-mediated excitotoxicity (116,120) energetic/mitochondrial stress (114,115) and oxidative insults (121).

In addition to sustaining BDNF levels, ECS treatment resulted in elevated levels of the protein chaperones Hsp70 and Hsp40, and the stress-responsive transcriptional regulator HSF-1, in the striatum of HD mice. Studies in which levels of Hsp70 or Hsp40 are selectively increased or decreased using molecular genetic technologies have shown that these two proteins can protect neurons against insults relevant to HD including excitotoxins, metabolic stress and mutant Htt (122,123). Moreover, Hsp70 can preserve electrical activity in striatal neurons subjected to mitochondrial stress (124), suggesting the possibility that therapeutic interventions that up-regulate Hsps may improve motor function in HD patients.

Mutant Htt causes major perturbations of energy metabolism as indicated by the negative energy balance and progressive weight loss of HD patients (125), demonstrated by mitochondrial/energy utilization deficits in brain (99) and peripheral (98,99) cells of HD patients, and by the dysregulated metabolic state of Htt mutant mice and striatal neurons therein (96,97,130). Recent findings suggest that reduced expression and/or impaired function of PGC-1α, which regulates mitochondrial biogenesis and function in neurons, plays a role in the abnormalities of cellular energy metabolism in HD (131). Thus, it was reported that mutant Htt impairs mitochondrial function, in part, by repressing PGC-1α gene transcription (132), and the global metabolic abnormalities in HD mice have been linked to perturbed PGC-1α actions (108). In addition, levels of PGC-1α target gene expression are reduced in skeletal muscle cells from HD patients and Htt mutant mice, and viral delivery of the PGC-1α increased expression of PGC-1α target
genes and resistance of HD myofibers to metabolic stress (133). We found that ECS treatment significantly attenuated the depletion of PGC-1α from striatal cells in HD mice. Therefore, it is likely that maintenance of PGC-1α contributes to process by which ECS treatment preserves mitochondrial function and protects neurons against mutant Htt.

Our preclinical findings demonstrate that ECS treatment counteracts the neurodegenerative process caused by mutant Htt in an animal model of HD. There has recently been a resurgence of interest in the use of ECS for the treatment of drug-refractive psychiatric disorders, and it is considered safe and effective for treatment of depression in the elderly, including those with co-morbidities (132). ECS treatments often result in long-lasting clinical improvements in psychiatric symptoms. We found that ECS treatment resulted in increases in the expression of several adaptive cellular stress response proteins (BDNF, Hsp70, Hsp40, and PGC-1α) which may promote neuronal survival and plasticity, and so forestall the neurodegenerative process resulting in a delay in the disease onset and life extension.

Neurodegenerative diseases, whether acute or chronic, are a tremendous medical problem in the modern world. Therapies are rare and only applied after a vast amount of neurons are lost due to the relatively late appearance of symptoms. The difficulty therein is early diagnosis and successful neuroprotection by targeting the pathological initiators early enough with effective pharmaceuticals to circumvent irreversible neuronal damage. HD is one of the most common inherited disorders in which there is progressive neurodegeneration and for which there is no cure. Because of strong genetic link and freely available genetic test, preventive therapy could be given to individuals who inherit the mutated htt gene and who will, at some stage, develop the disease. The opportunity to administer preventive therapy would maximize the time HD patients
spend in the mildest stages of the diseases, lessening movement disturbances and emotional cognition for the remainder of his or her lifetime.

There has been a resurgence of interest in ECS for the treatment of neurological disorders because it has evolved into a safe option and one that is effective. Furthermore, responsiveness to ECS does not abate with age which is the major risk factor for many neurological disorders. Recent studies on the neurobiological mechanisms of ECS reveal the induction of genes and molecules that lie at the crossroads of psychiatric and neurological disorders. We found that ECS increases the expression of BDNF, HSP70 and potentially other neuroprotective genes in the striatum and cortex, the major sites of cell loss in HD brain. The involvement of BDNF in cognitive processes and neuroprotection and the impaired BDNF expression reported for HD patients suggests that a deficit of this neurotrophin is a logical contributor to memory and cognitive problems and brain atrophy associated with the disease. ECS can provide additional benefits in the clinical management of psychiatric symptoms in HD patients, many of whom are refractory to medical treatment. Our findings provide a rationale for investigating further whether ECS could form the basis of an effective strategy to mitigate the neuropathological sequelae of gross brain and neuronal atrophy while preventing the development of dementia and psychiatric symptoms in individuals at risk for HD.

For future studies we would determine whether BDNF is essential for the beneficial effects induced by ECS, we will perform similar experiments in heterozygous BDNF KO mice. To combine ECS with pharmacotherapy to maximize treatment efficacy would be an efficient way to extend this therapy to the HD patients. As most existing drug treatments for HD produce only partial or moderate effects, we propose that combination treatment with ECS might prove to be a
more promising. We propose that combining safe and well-tolerated tricyclic antidepressants (TCAs) with ECS would have even greater efficacy and benefits by raising the levels of neuroprotective proteins in HD patients.


15. Emilie Colin, Diana Zala, Ge’ raldine Liot, He’ le’ne Rangone, Maria Borrell-Page, Xiao-Jiang Li, Fre´de´ric Saudou, and Sandrine Humbert. Huntingtin phosphorylation acts as a


19. Liliana B. Menalled and Marie-Françoise Chesselet. Mouse models of Huntington’s Disease. TRENDS in Pharmacological Sciences Jan 2002; .23(1); 32-9.


42. Josep M. Canals, Jose R. Pineda, Jesus F. Torres-Peraza, Miquel Bosch, Raquel Martin-Ibanez, M. Teresa Munoz, Guadalupe Mengod, Patrik Ernfors, Jordi Alberch1Brain-Derived Neurotrophic Factor Regulates the Onset and Severity of Motor Dysfunction Associated with Enkephalinergic Neuronal Degeneration in Huntington’s Disease. The Journal of Neuroscience, September 1, 2004; 24(35); 7727–7739.

44. Laurent R Gauthier, Bénédicte C Charrin, Maria Borrell-Pagès, Jim P Dompierre, Hélène Rangone, Fabrice P Cordelières, Jan De Mey, Marcy E MacDonald, Volkmar Leßmann, Sandrine Humbert, Frédéric Saudo. Huntingtin Controls Neurotrophic Support and Survival of Neurons by Enhancing BDNF Vesicular Transport along Microtubule. Cell 2004 Jul 9; 118(1); 127-38.


46. T. Milakovic, G.V.W. Johnson. Mitochondrial respiration and ATP are significantly impaired in striatal cells expressing mutant huntingtin. The J of Bio Chem. 2005; 280(35)2; 30773-82.

47. Lu-Shiun Her, Lawrence S. B. Goldstein. Enhanced Sensitivity of Striatal Neurons to Axonal Transport Defects Induced by Mutant Huntingtin. The Journal of Neuroscience, December 10, 2008; 28(50); 13662–13672.


50. Jean-Charles Lievens, Magali Iche, Monique Lava, Catherine Faivre-Sarrailh, Serge Birman. AKT-sensitive or insensitive pathways of toxicity in glial cells and neurons in Drosophila models of Huntington’s disease. Human Molecular Genetics 2008; 17(6); 882–894.


73. Petr Jezek, Lydie Hlavata. Mitochondria in homeostasis of reactive oxygen species


86. Songhee Jeon, Yong-Sik Kim, Joobae Park, Chang-Dae Bae. Microtubule affinity-regulating kinase 1 (MARK1) is activated by electroconvulsive shock in the rat hippocampus. Journal of Neurochemistry, 2005, 95, 1608–1618.


97. Andrew F. Neuwald and Tatsuya Hirano. HEAT Repeats Associated with Condensins, Cohesins, and Other Complexes Involved in Chromosome-Related Functions. Genome Res. 2000, 10: 1445-1452.


120. Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martín-Ibañez R, Muñoz MT, Mengod G, Ernfors P, Alberch J. Brain-derived neurotrophic factor regulates the


