HUNTINGTON’S DISEASE: A HARD NUT TO BREAK

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ABSTRACT
Huntington’s disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder, characterized by progressively worsening chorea, psychiatric disturbances, cognitive impairment and weight loss. The degenerative process primarily involves medium spiny striatal neurons (MSN) and to a lesser extent cortical neurons. Importantly, γ-aminobutyric acid (GABA)ergic projection neurons of the striatum, which make up roughly 90% of the striatal neurons are the most vulnerable in HD and their early dysfunction is responsible for the development of chorea. To date, there is no cure or clinically proven treatment that slows progression of this fatal disease. To investigate the mechanism of neurodegeneration in HD, animal models of HD have been generated using genetic manipulations, excitotoxins, and neurotoxins. Injections of NMDA receptor agonists, such as quinolinic acid, into the striatum, induce HD-like pathology, with a loss of projecting MSN and sparing of cholinergic and NADPH diaphorase neurons. Peripheral injections into rodents or primates of several mitochondrial toxins, including 3-nitropropionic acid (3-NP), also reproduce the aspects of behavioral, biochemical and striatal pathology found in HD

Keywords: Huntington’s diseases, 3-Nitropropionic acid, neurodegeneration

1.0. INTRODUCTION
Huntington’s disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder [1], characterized by progressively worsening chorea, psychiatric disturbances and cognitive impairment. Moreover, the degenerative process primarily involves medium spiny striatal neurons (MSN) and to a lesser extent cortical neurons. Importantly, γ-aminobutyric acid (GABA)ergic enkephalin neurons of the basal ganglia are the most vulnerable in HD and their early dysfunction is responsible for the development of chorea [2,3].

2.1 HUNTINGTON’S DISEASE
HD is caused by the expansion of a polymorphic (cytosine-adenine-guanine) CAG trinucleotide repeat encoding a polyglutamine tract within the Huntington protein [4], and the genetic defect is localized in the gene named IT15, on the short arm of chromosome 4 [5]. HD symptoms usually appear in middle age. However, disease can start earlier, and about 6% of HD patients develop juvenile forms [6].

Despite great progress, a direct causative pathway for the HD gene mutation to neuronal dysfunction and death has not yet been established [7]. However, recent evidence indicates that mutant huntingtin impairs fast axonal transport, and that this occurs prior to translocation of huntingtin into the nucleus [8,9,10]. The impairment of axonal transport might contribute to early neuropathology, and seems to precede transcriptional dysfunction, which is linked to the apoptosis and cell death that are characteristic of HD [5,9]. Because of early
functional decline and its genetic nature requires chronic and increasingly intensive multidisciplinary care. Therefore, HD disproportionately consumes medical, social and family resources [11].

2.2 HISTORY

Huntington's disease is a fatal neurodegenerative disease named after George summer Huntington, being the first who is credited with describing the disorder in 1872. His original paper published under the title “On Chorea” is brief, complete and still up to date in major respects. He presented his findings before the Meigs & Mason Academy of Medicine at Middleport, Ohio on 15th February 1872 and published it only two months later in Philadelphia Journal, The Medical and Surgical Reporter [12]. The great American folk singer and composer Woody Guthrie died with complications of HD on October 3, 1967. The death of Woody Guthrie led to the foundation of the Committee to Combat Huntington's Disease. Dr. Milton Wexler in 1968, after experiencing HD in his wife's family, was inspired to start the Hereditary Disease Foundation (HDF), with the aim of curing genetic illnesses by coordinating and supporting research [13].

2.3 SYMPTOMS AND STAGES OF HD

The symptoms of HD vary widely from person to person, even within the same family. For some, involuntary movements may be prominent even in the early stages. For others, these may be less evident and emotional and behavioral symptoms may be more obvious. Symptoms of HD begin insidiously, most commonly between the ages of 35 and 50, but the age of onset can vary from early childhood until old age [14,15]. The disease is relentlessly progressive and fatal 15-20 years after the onset of symptoms [16,17]. The genetic mutation underlying HD was discovered in 1993 [5]. HD is caused by the inheritance of an unstable and excessively repeated cytosine-adenine-guanine codon within the coding sequence in exon 1 of the IT-15 gene [5]. Unaffected individuals were found to have repeats with lengths of 34 CAG's or less, while HD affected individuals had anywhere between 40 to 121 repeats. In HD, the severity of symptoms seems to be correlated with the number of repeats while the age of onset appears to be inversely proportional to the repeat amount [18,19]. Juvenile variant of the disease (with an early onset) may exhibit up to 250 repeats.

THE FOLLOWING ARE THE COMMON FEATURES OF HD:

2.3.1 MOTOR SYMPTOMS

The movement disorder of HD consists of two components: involuntary movements and abnormal voluntary movements. Chorea, or choreoathetosis, is the movement abnormality most frequently associated with HD [20]. As the disease progresses, choreiform movements may be reduced in intensity or frequency; the initial hyperkinetic syndrome being progressively replaced by a more hypokinetic syndrome in which bradykinesia, rigidity and dystonia dominate [21,22].

2.3.2. COGNITIVE/INTELLECTUAL SYMPTOMS

Cognitive impairment occurs in HD patients which are due to degeneration of brain cortex neurons. Patients have significant problems with frontal executive functions, such as problems with maintenance, cognitive flexibility, abstraction, judgment [23,24], reasoning, sequencing, organizing, planning, and adapting [25,26]. Working memory may be affected in patients with HD because of frontal lobe dysfunction associated with the bidirectional connections with the caudate [27].

2.3.3. EMOTIONAL/BEHAVIORAL SYMPTOMS

Depression, irritability, anxiety and apathy, obsessive-compulsive disorder, psychosis, paranoia, and substance abuse are often encountered in HD [28,29]. Behavioral changes may include aggressive outbursts, impulsiveness, mood swings and social withdrawal, feelings of worthlessness or guilt, self-blame, changes in sleep and appetite, anxiety, anhedonia, loss of energy, hopelessness, and diurnal variation of mood with more severe symptoms [30].
2.3.4. WEIGHT LOSS

It is a fourth characteristic of the disease and may be due to dysphagia as well as degeneration of hypothalamic orexin-positive neurones [31,32].

2.3.5. LINK BETWEEN CHOREA AND STRIATAL DEGENERATION

FIGURE 1 - BASAL GANGLIA AND ASSOCIATED BRAIN AREAS (TORTORA GJ & DERRICKSON, 2009)

The primary motor dysfunctions in HD are due to the degeneration of a subset of striatal neurons [33,34]. The striatum belongs to a system called basal ganglia which are a collection of subcortical nuclei that are involved in the control of movement (Fig. 1). The basal ganglia do not receive direct sensory input and send little output to the spinal cord. The major flow of information arrives from the cortex into the striatum and returns to the cortex through the thalamus, which is the major output structure of the basal ganglia. A classical model developed by Albin et al. (1989) described two major neuronal motor pathways, which act in opposition, and correctly predicts the motor impairment in HD. The ‘direct pathway’ (Fig. 2) promotes movements by a relay of two inhibitory synapses, first in the globus pallidum internal segment and the substantia nigra pars reticulate (Gpi/SNpr) and second in the thalamus [36,37]. The ‘indirect pathway’ (Fig. 2) inhibits movement by three inhibitory connections in the globus pallidum external segment (GPe), the substantia nigra pars compacta (SNpc) and the thalamus with a relay of GABAergic inhibition between the SNpc and SNpr [38,39]. A more recent view of the basal ganglia functions indicates that in addition from the striatum, the subthalamic nucleus also receives input from the cortex [40,41] and sends output to the Gpi/SNpr. This new cortico-subthalamo-pallidal loop is called ‘hyperdirect pathway’ and acts by inhibiting the thalamus and cortex. As this loop is not modified in HD, the model proposed by Albin et al (1989) correctly predicts the motor defects present in Huntington’s disease [42].

FIGURE 2- SCHEMATIC WIRING DIAGRAM OF THE BASAL GANGLIA (BRUNTON ET AL., 2006)
2.3.6 Neuropathology of HD

All humans have the Huntingtin gene (HTT), which codes for the protein Huntingtin (htt) [43]. Proteolytic degradation of the protein Huntingtin (htt) generates N-terminal fragments that are released into the extracellular matrix. These fragments are prone to aggregation, and, while their toxicity is debated, the aggregation of htt is probably an important factor in HD pathology. It has been shown that htt aggregates sequester transcription factors, such as CREB-binding protein, thereby disrupting transcription of essential genes by inhibiting the arrangement of downstream factors and counterparts and thus cellular signaling [44,45,46].

Interestingly, several other polyQ disorders seem to also affect the CREB transcriptional pathway [44]. N-terminal htt fragment aggregates are recognized by the body as misfolded proteins. Normally, molecular chaperones work to refold the proteins and if this is unsuccessful, then the aggregates are cleared by the ubiquitin-protease system (UPS). However, in HD the rate of misfolding and aggregation seem to overwhelm the cells UPS. It has been proposed that the failure of the UPS is necessary for the pathogenesis of HD [47]. The inability of the UPS to degrade the large stretches of glutamine may result in the release and aggregation of N-terminal fragments. Furthermore, the sequestration of molecular chaperones into the N-terminal aggregates reduces their availability to other physiological systems [48]. Thus, vital methods of cellular waste management are disrupted [44,45,46].

As previously mentioned, the absence of wild-type Htt also plays a role in the disease pathogenesis. The production of Brain-derived neurotrophic factor (BDNF) is controlled by the transcription factors Repressor Element-1-Silencing transcription Factor/ Neuron Restrictive Silencer Factor (REST/NRSF), which halt the production of this growth factor [49]. Normally, wild-type htt binds to the REST/NRSF complex and prevents them from antagonizing the transcription of BDNF. The mutant polyQ expanded form of Htt is unable to properly bind the REST/NRSF complex resulting in a profound reduction of BDNF transcription and translation. This reduction of the neurotrophic factor BDNF may in part underlie the death of the spiny-projection neurons of the striatum, as they are especially reliant on this factor [44,45,50].

2.4 Biochemical Alterations in HD Brains

Biochemical alterations found in the caudate of patients with HD are a consequence of selective neuronal cell death from basal ganglia. These changes include decreased levels of GABA and its synthesis enzyme glutamate decarboxylase (GAD), acetylcholine (ACh) and its synthesis enzyme choline-acetyl transferase (ChAT), some peptides specifically localized in middle-size spiny neurons [51,52,53] along with important alterations in the number of NMDA receptors [54,55]. Activities of succinate dehydrogenase (SDH) and cytochrome oxidase (components of complexes II-III and IV of the electron transport chain, respectively) are markedly reduced in advanced grade HD caudate and putamen, but are unaltered in other brain regions [56,57]. Various biochemical alterations in HD are summarized in (Fig. 3).

![FIGURE 3 - BIOCHEMICAL ALTERATIONS IN CAUDATE OF HD PATIENTS [51].](image-url)
2.5 PATHOLOGICAL FEATURES OF HD
2.5.1 MECHANISMS OF NEURODEGENERATION:
THE LETHAL TRIPLET

There are three main mechanisms of neuronal cell death, which may act separately, or cooperatively to cause neurodegeneration. This lethal triplet of metabolic compromise, excitotoxicity, and oxidative stress [58], causes neuronal and cell death that is both necrotic and apoptotic in nature [59,60,61].

In general, necrosis is a form of cell death by 'murder' that involves an injury from which the cell cannot recover which causes it to subsequently lyse, while apoptosis is an active program of cell 'suicide' whereby the cell activates (or is forced to an injurious stimulus) a self-destruct mechanism which causes it to shrink and ultimately be phagocytosed by microglia [62].

2.5.1.1 METABOLIC COMPROMISE OF NEURONS

The synthesis of ATP for energy in neurons occurs mainly in the mitochondria via the TCA cycle and the electron transport chain, therefore mitochondrial poisoning results is a depletion of ATP, which can be cytotoxic [63,64]. Mitochondrial toxin 3-NP causes cytotoxicity restricted mainly to the striatum when administered systemically to rats despite the fact that metabolic impairment actually occurs throughout the entire body and brain [65,66]. Dysfunction of mitochondria results in loss of intracellular calcium (Ca$^{2+}$) buffering capacity and increase in the production of damaging oxygen nitrogen free radicals, leading to oxidative and nitrosative stress, respectively. These free radicals further enhance 3-NP-induced cytotoxicity [67,68,69].

It is well documented that the antioxidant enzyme superoxide dismutase (SOD), the endogenous antioxidant glutathione (GSH), [70,71] the anti-apoptotic signal bc-2, and the pro-apoptotic enzymes the caspases all function in the mitochondrial inner membrane are disrupted due to mitochondrial dysfunction [72]. Meanwhile, the depletion of ATP causes failure of ATP-dependent ion pumps, which results in depolarization of neurons [73]. This results in a loss of ionic integrity and an accumulation of intracellular Ca$^{2+}$. The accumulation of Ca$^{2+}$ in mitochondria rather than in the cytoplasm may be more critical in determining cell death [74]. This intracellular Ca$^{2+}$ then induces further mitochondrial strain, free radical generation, and a host of downstream neurotoxic processes such as activation of Ca$^{2+}$-dependent proteases and lipases [58].

2.5.1.2 EXCITOTOXICITY

Neuronal excitotoxicity usually refers to the death of neurons arising from prolonged exposure to glutamate and associated excessive influx of ions and water into the cell. The resulting calcium overload is particularly neurotoxic, leading to activation of enzymes that degrade proteins, membranes and nucleic acid [75]. Excitotoxicity in HD may be the result of a reduced threshold for the glutamate toxicity that would occur in neurons with compromised energy metabolism, in which otherwise normal levels of excitatory neurotransmitter to become toxic. One of the hypotheses that has been put forth to explain the exquisite sensitivity of the medium spiny projection neurons of the striatum to degeneration in HD is the "excitotoxicity hypothesis". The hypothesis stipulates that excessive activation of glutamate receptors, which may be due to increased glutamate release from cortical afferents, reduced uptake of glutamate by glia or hypersensitivity of post-synaptic glutamate receptors on striatal projection neurons, likely in combination with pathological signaling downstream of glutamate receptor activation arising from altered intracellular calcium homeostasis and mitochondrial dysfunction, would result in neuronal dysfunction and death of striatal MSNs [76,77].

Excitotoxic lesioning of the striatum with ligands for the NMDA glutamate receptor subtype produces a pattern of neuropathology which mimics that seen in human post mortem HD striatum. Abundant evidences show that the toxic effects of excitatory amino acids (EAAs) are mainly due to the activation of the NMDA receptor which leads to an influx of Ca$^{2+}$ and then toxic overloading [73]. Ca$^{2+}$ overloading can cause the indiscriminate activation of calcium-dependent signals such as phospholipases and proteases, as well as oxidative stress through reactive oxygen species (ROS) and reactive nitrogen species (RNS) [78].
cytotoxicity of Ca\(^{2+}\) is a complex mechanism and may involve not only Ca\(^{2+}\) overloading but also disordering of intracellular Ca\(^{2+}\) dynamics and mitochondrial ATP synthesis [75]. NMDA has been shown to increase not only cytosolic levels of Ca\(^{2+}\) but also mitochondrial Ca\(^{2+}\) concentration [79].

### 2.5.1. iii OXIDATIVE STRESS

Oxidative stress is due to the actions of highly reactive free radicals such as the ROSs superoxide anion (O\(_2^-\)) and hydroxyl radical (OH\(^-\)), and the RNS peroxynitrite (ONOO\(^-\)) [80,81]. The oxidizing actions of these reactive species destroy membrane lipids, proteins and DNA and can be detrimental to cells if they accumulate at high levels or if deficits in cellular antioxidant defense systems occur. ROS and RNS are generated under normal cellular functioning, mainly during mitochondrial respiration, and are deactivated by endogenous antioxidants and scavengers [82]. 3-NP may enhance the production of toxic free radicals, such as intracellular Ca\(^{2+}\), dopamine (DA), and inducible nitric oxide, which can then react with superoxide anion to form peroxynitrite, causing mitochondrial depolarization and depletes ATP [78].

### 2.6 ANIMAL MODELS OF HUNTINGTON'S DISEASE

Animal models which closely mimic the neurobiological and clinical symptoms of the disease may provide an alternative approach for the study of HD molecular pathogenesis, the refinement of existing treatments and the development of novel therapies for HD. Many animal models mimic HD symptoms or pathology. Characterization of multiple animal models is necessary for understanding the pathogenesis identification and characterization of novel targets, development of new drug and treatment strategies, and effects of potential drugs acting through known mechanisms. Broadly animal models of HD can be classified into three major categories:

1) **Excitotoxic models**

2) **Impaired energy metabolism models**

3) **Transgenic models**

#### 2.6.1. EXCITO TOXIC MODELS

Before the emergence of genetic models, different toxins were delivered to rodents and primates to reproduce a HD-like phenotype [21,83]. Excitotoxicity involves a drastic increase in intracellular Ca\(^{2+}\) concentrations in response to an over-exposure of neurons to the effects of excitatory amino acids (EAA) [27], such as glutamate and its analogues. NMDA receptors play a relevant role in the neurotoxicity induced by EAA. The overstimulation of glutamate receptors (NMDA) with excitatory amino acids such as ibotenic acid, kainic acid, NMDA or quinolinic acid induces neuronal death by excitotoxicity [84,85,86]. A sudden and drastic influx of the ion onto the neuronal cell may trigger metabolic lethal pathways involving proteolytic enzymes, calpain, protein kinase C, DNAase, phospholipase and can induce cell death either by necrosis or apoptosis [78] thus increasing the formation of ROS and RNS [87,88]. Quinolinic acid and kainic acid have been investigated in the rat brain with the aim to compare the extent of neuronal damage that they produce when injected in rat brain [89,90,91]. Morphologic alterations and biochemical profiles in the striatum resembling alterations in HD brains such as decreased levels of GABA, ACh and substance P, as well as selective axon-sparing lesions were observed [92,93]. However, quinolinic acid exerted more selective and specific effects similar to those of HD, as it produced no major changes in somatostatin, neither in neuropeptide Y [94,95].

Recent findings demonstrating that the levels of quinolinic acid, together with those of another kynurenine metabolite is responsible for production of ROS, 3-hydroxykynurenine, are increased in the striatum and cortex of HD brains [97,98]. Such evidence has established the formal concept of the “kynurinergic hypothesis.” The kynurenine pathway is a metabolic route typically located in glial cells and reduces tryptophan into several metabolites with different redox functions [98]. Quinolinic acid is an endogenous metabolite at the kynurenine pathway acting as a selective agonist at the NMDA receptor [99,100], and produces membrane depolarization and calcium influx into neuronal cells, resulting in damage of nerve cells due to oxidative stress, apoptosis, and...
necrosis [101]. Nuclear factor kappa B (NF-κB) activation is also contributing to excitotoxin-induced death of striatal neuron [102,103]. Some regulatory proteins such as Bcl-2, p53 and c-Myc contribute to the differential vulnerability of striatal neuron to the NMDA receptor agonist quinolinic acid [104]. Further, excitotoxin injection into brain causes microglial activation and the expression of pro-inflammatory cytokines and iNOS and COX-2 which enhance ROS, RNS, and oxidative stress that showed considerable increase in oxidative damage markers 4-hydroxynonenal (4-HNE) and 8-OHdG of striatum [105]. It has been reported that quinolinic acid reduces the antioxidant defenses in rats [106].

2.6.2 ENERGY METABOLISM DEFICIT MODEL

CNS exhibits selective vulnerability in energetic resource due to elevated metabolism of neuronal cells, therefore any alteration in energy metabolism represent a potential risk for loss of neuronal viability [107]. In HD, decreased levels of glucose and oxygen in basal ganglia and brain cortex have been reported [33]. It is well known that energy metabolism failure leads to cell death. Different animal model have been designed using various mitochondrial toxins like amino-oxyacetate, rotenone, MPP⁺ malonate, Mn²⁺, 3-acetyl-pyridine and 3-nitropropionic acid (3-NP) into the rat striatum that produce increased lactate formation, ATP-depletion and neuronal degeneration by mechanism of disrupting mitochondrial energy metabolism and secondary excitotoxicity [108,109,110,111,112]. 3-NP causes mitochondrial damage by inhibiting SDH, an enzyme involved in both the tricarboxylic acid and the electron transport chain [113,114,115]. Researchers have replicated this finding in rats [116], mice [117], and nonhuman primates [83,118,119]. 3-NP crosses the blood-brain barrier and can be administered systemically to rats, mice, and nonhuman primates [120].

2.6.3. TRANSGENIC MOUSE MODELS OF HD

An important advance in the study of HD was the development of transgenic mouse models. The ideal transgenic mouse model would have a robust phenotype, rapid disease onset and progression, well-defined behavioral abnormalities that can be quantified, and neuropathological features such as selective loss of striatal projection neurons, all of which accurately replicate human HD. There are many mouse models of HD and they fall into three broad categories: (1) mice that express exon-1 fragments of the human huntingtin gene containing polyglutamine mutations (in addition to both alleles of murine wild-type huntingtin, Hdh); (2) mice with pathogenic CAG repeats inserted into the existing CAG expansion in murine Hdh (knock-in mice); and (3) mice that express the full-length human HD-gene (plus murine Hdh).

2.7 3-NITROPROPIONIC ACID-INDUCED NEUROTOXICITY: AN ANIMAL MODEL OF HUNTINGTON'S DISEASE USED IN PRESENT STUDY

3-NP, a metabolite of 3-nitropropanol, is a toxic agent produced by certain plants and fungi of Arthrinium and Aspergillus genus [121,122,123]. The fatalities after eating moldy sugarcane have been linked to 3-NP toxicity [124]. The first initial reports of livestock poisoning by 3-NP came from western U.S.A. [125]. Animals intoxicated with leguminous plants presented various motor abnormalities consisting of general weakness and dis-coordination of hind limbs evolving to paralysis [22,118]. The 3-NP injection could produce basal ganglia degeneration and is a specific inhibitor of mitochondrial respiratory complex II SDH, an enzyme located in the mitochondrial inner membrane [33,34,126]. Prior subacute 3-NP poisoning seems to provide resistance to ischemic damage to nervous tissue by a preconditioning effect [127] similar to that resulting from mild ischemia. Because of the behavioral and histological similarities with HD, this model is highly validated and well established animal model of Huntington's disease [83,114]. Chronic 3-NP induced lesions have striking similarity with HD. This is considered to be a relevant phenotypic experimental model for the study of Huntington's disease [21,128]. The target of 3-NP-Complex II is both a member of the Krebs tricarboxylic acid cycle (oxidizing succinate to fumarate) and an entry-point for electrons into the respiratory chain at the level of ubiquinol [69,129,130]. It consists of a large flavoprotein subunit (FP) containing covalently bound FAD,
iron-sulfur protein (IP) with three different iron-sulfur clusters, and two small membrane anchor subunits (chains C and D) ligating a single low-spin heme of type B (Fig.4)  

2.7.1 MITOCOCHONDRIAL IMPAIRMENT  

Mitochondria are the energy powerhouses and buffering sinks of the cell not only are they the sites of oxidative phosphorylation and cellular respiration in the generation of ATP, but they also play a critical role in the maintenance of a low concentration of calcium within the cytosol [131]. Change in either of these critical functions of mitochondria have dire consequence and often determine the cell fate in survival/death signaling pathway. 3-NP dysregulates mitochondrial function and this metabolic impairment involve three interacting processes: energy impairment, oxidative stress and excitotoxicity [58,65, 115,132]. 3-NP, result in ATP-depletion which impairs intracellular calcium buffering capacity and leads to the production of damaging oxygen and nitrogen free radicals [86,132,133,134].  

Electrons from NADH and FADH$_2$ enter respiratory chain at, respectively, complex I and II. Complexes I, III, and IV function as proton pumps, in series with respect to protons and in parallel with respect to proton circuit. Proton reentry can occur through ATP synthase to generate ATP and also through transhydrogenase (TrH) to reduce NADP1.

These free radicals further enhance 3-NP-induced neurotoxicity [67,68,69]. 3-NP may also elicit secondary glutamate excitotoxicity, neuronal death as a result of chronic exposure to the excitatory amino acid glutamate [73,135]. It is well documented that the antioxidant enzyme SOD, the endogenous antioxidant glutathione (GSH) [70,71], the anti-apoptotic signal bcl-2, and the pro-apoptotic enzymes the caspases all function in the mitochondrial inner membrane and disrupted due to mitochondrial dysfunction [72,136,137]. Meanwhile, the depletion of ATP causes failure of ATP-dependent ion pumps, which results in depolarization of neurons [73,138,139]. This results in a loss of ionic integrity and an accumulation of intracellular Ca$^{2+}$. The accumulation of Ca$^{2+}$ in mitochondria rather than in the cytoplasm may be more critical in determining cell death [74,140]. This intracellular Ca$^{2+}$ then induces further mitochondrial strain, free radical generation, and a host of downstream neurotoxic processes such as activation of Ca$^{2+}$-dependent proteases and lipases [58,87].  

3-NP has been shown to readily penetrate into the brain, inactivating SDH activity within the first 2 hr following intraperitoneal (i.p.) injection and significantly inhibiting SDH activity for at least 24 hr [21]. Furthermore, the 3-NP-induced striatal lesions observed following chronic 3-NP intoxication were associated with a 50-60% SDH inhibition, a level of enzyme inactivation similar to the degree of complex II-III deficiency observed post-mortem in the HD caudate nucleus [141,142,143]. However, a lower level of inhibition probably produces functional changes without actual neurodegeneration and progressive aggravation of the inhibition of SDH activates several biochemical pathways involved in cell death and/or survival [124,144].  

FIGURE 4 - NEUROTOXIN SITE IN RESPIRATORY CHAIN [21]
2.7.2 EXCITOTOXICITY

Excitotoxicity in HD might be the result of a reduced threshold for glutamate toxicity that would occur in neurons with a compromised energy metabolism, in which otherwise normal levels of this excitatory neurotransmitter become toxic [145,146]. NMDA receptors are highly permeable to calcium ion, change in NMDAR activity and or function invariably result in changes to intracellular calcium levels. Altered calcium levels in turn affect downstream effectors pathway and initiate second messenger cascades causes buffering capacity for calcium be exceeded or the compartmentalization of intracellular calcium perturbed, these pathway will lead to the activation of catabolic enzymes, such as nucleases, proteases and phospholipases, as well as generation of free radicals and induction of mitochondrial damages [75]. Cellular dysfunction and death eventually follow. 3-NP causes uptake of calcium into the mitochondrial matrix results in moderate depolarization of the inner mitochondrial membrane, which generally recovers following removal of the stimulus and a return towards baseline cytosolic calcium concentration. However, during a toxic glutamate stimulus, excessive loading of calcium into mitochondria can activate the mitochondrial permeability transition (mPTP) resulting in massive depolarization of the mitochondrial inner membrane, dumping of calcium back to the cytosol and release of factors that activate caspase-9 and -3 as well as the induction of the apoptotic death program [72], and abolishing oxidative generation of ATP [69,111]. Moreover uptake of calcium into mitochondria is required for NMDAR-mediated excitotoxicity [74], which leads to striatal medium spiny neurons (MSNs) degeneration in HD [69,147]. In HD causes activation of apoptotic factors via mitochondrial inhibition like as cytochrome c, Smac/Diablo, apoptosis-inducing factor, and endonuclease G. 3-NP causes release of apoptotic factor that is cytochrome C that binds to Apaf-1 and together with procaspase 9, forms the "apoptosome", which activates caspase-9. This enzyme activates procaspase-3 which leads to activation of caspases-3 [59,148,149,150,151].

2.7.3 GENERATION OF FREE RADICAL OXIDATIVE DAMAGE

Oxidative damage is due to the actions of highly reactive free radicals such as the ROSs superoxide anion (O2−) and hydroxyl radical (·OH), and the RNS peroxynitrite (ONOO−) [80,81,134,152]. The oxidizing actions of these reactive species destroy membrane lipids, proteins and DNA and can be detrimental to cells if they accumulate at high levels or if deficits in cellular antioxidant defense systems occur [153]. ROS and RNS are generated under normal cellular functioning, mainly during mitochondrial respiration, and are deactivated by endogenous antioxidants and scavengers [82,115]. 3-NP also enhances the production of toxic free radicals, such as intracellular Ca2+, dopamine (DA), and inducible iNOS [154,155,156]. Activation of intracellular Ca2+ lead activation of NOS results in the increased formation of NO, which can then react with superoxide anion to form peroxynitrite anion, while superoxide anion may spawn the production of hydrogen peroxide through action of manganese superoxide dismutase (MnSOD) [82]. Under physiological condition hydrogen peroxides, but if it is formed excess, it can react with transition metal ion (Fe2+ and Cu2+) in the fenton reaction, to generate the other highly reactive toxic radical, hydroxyl radical that causes mitochondrial depolarization DNA damage and depletes ATP [157]. Also, peroxynitrite can activate the enzyme, PARP, plays a role in DNA repair by ADP-ribosylating nuclear proteins at the sites of DNA nicks to facilitate DNA repair. If DNA damage is excessive, then PARP activation exhausts supplies of its cofactor NAD+ (which donate the ADP-ribose group). ATP is then required to replenish NAD+ levels and this can lead to depletion of ATP and energy compromise [158]. The oxidation of membrane lipids (lipid peroxidation) by free radicals produces a cytotoxic byproduct, HNE, which has recently been identified as a mediator of oxidative stress induced neuronal cell death [130,159]. HNE impairs glucose transport which can lead to energetic failure. HNE also renders neurons more sensitive to excitotoxicity as it inhibits Na+/K+/ATPase activity which is necessary for maintaining neuronal polarization and therefore the voltage-dependent Mg2+ block of the NMDA receptor channel [66]. Furthermore, the use of well-
known endogenous and exogenous antioxidants, such as coenzyme Q10, N-acetylcysteine, melatonin, S-allylcysteine, resveratrol, curcumin and dehydroepiandrosterone, results in protective effects against the neuronal damage induced by the toxin [27,66,69,160,161,162]. Although the precise mechanisms by which antioxidants may protect against 3-NP toxicity still remain unclear, these effects might be related either with scavenging of free radicals leaked from the altered chain transport, or with preventive actions on blockade of SDH. Nevertheless, when taken together, all these evidences are pointing to the key role of oxidative stress in the pattern of toxicity elicited by 3-NP leading to neurodegeneration [163].

2.7.4 INFLAMMATION

Microglia is the major intrinsic immunocompetent phagocyte cells in the CNS. They comprise 10–20% of the white cell population and are normally found in a quiescent state with spidery processes. The term "glia" derived from the Greek word for "glue," suggests that microglia share with astroglia and oligodendroglia the property of brain support and, more particularly, the support of neurons. However, such a supportive role in the healthy brain is better appreciated for astroglia, which make important contributions to neurotransmitter metabolism, and for oligodendroglia, which are the source of myelin, than for ramified (resting) microglia. Upon exposure of the brain to any form of insult, such as trauma, infection or ischemia, the microglia rapidly become activated [166,167].

3-NP causes activation of microglia and astroglia which produce cytotoxic substances including pro-inflammatory cytokines such as TNF-α and IL-1β [126,165], via activation of MAPkinase and activation of NF-κB [164,168]. These cytokines in turn cause further activation of microglia, resulting in a self-propagating inflammatory cascade leads to neurodegeneration [165] (Fig. 5). Activated microglia-mediated neurotoxicity was found to be linked to caspase-3 activation and leads to apoptosis [169]. It is well known that activated microglia also release excitotoxins such as glutamate and quinolinic acid, and contribute to NMDA-mediated excitotoxicity, involved in neuronal death [170]. Further 3-NP also causes production of NO and PGs which ultimately leads to nitrosative stress and oxidative stress, leads to neuronal death [27,111,162,163]. 3-NP is also involved in activation of microglia and astroglia which activates NF-κB [164] and phosphorylation of IκBs by kinase complex known as IκB kinase (IKK). These processes allow the translocation of NF-κB into nucleus and production of pro-inflammatory cytokines which leads in neuronal death [111,165,168].

2.7.5 3-NP ANIMAL MODEL OF HD-LIMITATION AND ADVANTAGES

Despite chronic 3-NP rat models replicating some of the features of HD, this model still has some limitations. Primarily, there is a very different...
reertoire of movements in the primate, as compared to the rat [171,172]. Secondly, the organization of the basal ganglia in primates is quite different from that in rats [173]. In primate animals, but not in rats, the striatum is structurally divided into 2 parts: the caudate nucleus and the putamen. This difference between the rodent and primate models is exemplified by the behavioral response to the dopamine agonist apomorphine observed in non-human primates with excitotoxic-induced striatal lesions [174,175]. In non-human primates with chronic 3-NP treatment, a variety of abnormal movements are highly reminiscent to those seen in HD patients; these types of movements have never been observed in rats in the same experimental conditions [173]. As discussed earlier, rats chronically treated with 3-NP did not show clearly identifiable dyskinetic movements resembling chorea even though hyperlocomotor activity has been reported early in the course of intoxication, as well as the presence of dystonia, bradykinesia and gait abnormalities [176,177]. Therefore, it may be that the dyskinetic component of HD symptomatology is part of a motor repertoire that can only be expressed in primates.

The importance of this model is that chronic systemic administration of 3-NP produces motor dysfunctions and striatal lesions that mimic many histological and neurochemical features of HD [178,179]. These models also offer the flexibility to investigate HD at different stages. Moreover, the efficacy of experimental treatments can be tested at various times of disease progression. The use of mitochondrial toxin lesion models have led to the clinical administration of mitochondria function protectors, including coenzyme Q10 and creatine for the treatment of HD. However, many studies have shown that these mitochondria function protectors only have limited beneficial effects for HD patients [180]. Since the mutant gene that causes HD was identified and numerous genetic animal models have been generated. Because genetic models can mimic the pathology of HD more accurately, chemical lesion models are now considered outdated. However, these models still have some validation. For example, excitotoxin and mitochondrial toxin are often used today in transgenic HD models and other in vitro models to study the sensitivity of genetic models to these toxins [181].

2.8. THERAPY (CURRENT THERAPEUTIC OPTIONS)

Various therapeutic targets for HD (Fig. 6) are being focused to develop neuroprotective agents that will slow or halt the course of the illness [182]. Because there are no effective neuroprotective therapies that delay the progression of the disease, symptomatic treatment remains the cornerstone of medical management. Several classes of medications have been used to ameliorate the various symptoms of HD, including typical and atypical neuroleptics, dopamine depleters, antidepressants, antiglutamatergic drugs, GABA agonists, antiepileptic medications, acetylcholinesterase inhibitors, and botulinum toxin. Recently, surgical approaches including pallidotomy, deep brain stimulation, and fetal cell transplants have been used for the symptomatic treatment of HD [183].

FIGURE 6 - DOWNSTREAM TARGETS FOR HUNTINGTON'S DISEASE (HD). EVIDENCE FROM THE TRANSGENIC MOUSE MODEL OF HD INDICATES THAT THESE PROCESSES INFLUENCE NEURONAL DEATH (182).
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