Brain Pathology 9: 147-163(1999)

SYMPOSIUM: Oxidative Stress in Neurological Disease

Oxidative Stress in Huntington's Disease

Susan E. Browne[1], Robert J. Ferrante[2], M. Flint Beal[1,3]

[1] Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston MA
[2] Bedford VA Medical Center, Bedford MA, and Departments of Neurology, Pathology, and Psychiatry, Boston University School of Medicine, Boston, MA
[3] Department of Neurology, New York Hospital and Cornell University Medical College, New York NY.

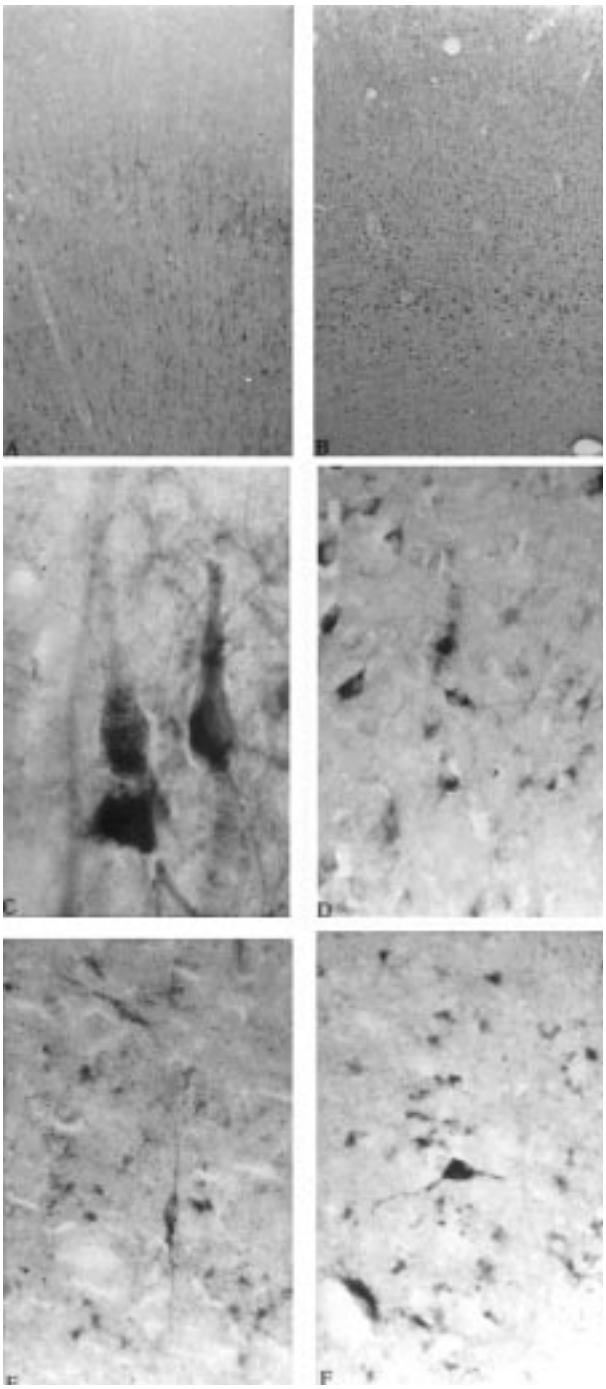
It has been five years since the elucidation of the genetic mutation underlying the pathogenesis of Huntington's disease (HD) (97), however the precise mechanism of the selective neuronal death it propagates still remains an enigma. Several different etiological processes may play roles, and strong evidence from studies in both humans and animal models suggests the involvement of energy metabolism dysfunction, excitotoxic processes, and oxidative stress. Importantly, the recent development of transgenic mouse models of HD led to the identification of neuronal intranuclear inclusion bodies in affected brain regions in both mouse models and in HD brain, consisting of protein aggregates containing fragments of mutant huntingtin protein. These observations opened new avenues of investigation into possible huntingtin protein interactions and their putative pathogenetic sequelae. Amongst these studies, findings of elevated levels of oxdative damage products such as malondialdehyde, 8-hydroxydeoxyguanosine, 3-nitrotyrosine and heme oxygenase in areas of degeneration in HD brain, and of increased free radical production in animal models, indicate the involvement of oxidative stress either as a causative event, or as a secondary constituent of the cell death cascade in the disease. Here we review the evidence for oxidative damage and potential mechanisms of neuronal death in HD.

Corresponding author:
Susan E. Browne, Ph.D., Neurology Research, Warren 408, Massachusetts General Hospital, Fruit Street, Boston, MA 02114; Tel.: (617) 726-8463; Fax: (617) 724-1480
E-mail: browne@helix.mgh.harvard.edu

Introduction

The late onset and progressive development of the behavioural abnormalities, cognitive impairment and involuntary choreiform movements which characterize Huntington's disease (HD) are the physical manifestations of a mutation in a gene on chromosome 4 encoding so-called "huntingtin" protein. The first clinical symptoms of HD are generally psychiatric abnormalities, most commonly depression and mood disturbances, which typically present in the fourth or fifth decade of life. Involuntary choreiform movements and dementia develop over the next 15-20 years, and death generally results from complications of immobility. This autosomal dominantly inherited degenerative disorder is characterized neuropathologically by bilateral striatal atrophy with marked neuronal loss and astrogliosis within the caudate and putamen. Within the striatum, spiny projection neurons containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) are particularly vulnerable to degeneration in HD, whilst large cholinergic interneurons and aspiny interneurons containing nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), somatostatin and neuropeptide Y appear to be relatively spared (46, 39, 81, 82). Less severe neuropathological changes also occur in other brain regions, notably cerebral cortex, at more advanced stages of the disease.

Despite identification of the genetic abnormality in HD (97), resulting in expression of an expanded polyglutamine tract in huntingtin protein, the definitive role of mutant huntingtin in neuronal degeneration remains unclear. As discussed below, the insidious progression of motor and behavioural disturbances in HD reflect the selective pattern of cell loss in the brain, and the specific neurotransmitter pathways affected. The reason for the preferential vulnerability of striatal neurons is also unknown at present, and cannot be simply explained in terms of the distribution of abnormal huntingtin since the gene mutation is expressed throughout the body. However, experimental evidence suggests that the



pathogenesis of cell death in HD is linked to a gain of function of mutant huntingtin, and involves energetic defects, oxidative damage and excitotoxic processes. In this review we summarize the current understanding of neuropathological events in HD brain and discuss putative pathogenetic mechanisms, with particular emphasis on the role of oxidative damage in cell death. We will address three broad themes: 1) Neuropathological changes in HD; 2) Functional alterations in HD brain; and 3) Potential mechanisms linking functional alterations with their pathological sequelae in HD.

Neuropathological Changes in HD

Gross Neuropathological Features of HD. Motor dysfunction in HD results from the disruption of basal ganglia-thalamocortical pathways regulating movement control. The primary site of neuronal loss and atrophy in HD brain is in the caudate-putamen, although in many cases atrophy also occurs in a number of non-striatal regions, including cerebral cortex, thalamus, globus pallidus (GP), cerebellum and white matter tracts (31, 99). The typical neuropathological features of HD have recently been thoroughly and definitively outlined in a review by Vonsattel and DiFiglia (100), and therefore will be addressed only briefly here. In summary, the typical pattern of striatal cell loss in HD occurs gradually along a topographically well defined pathway (31, 99). Neurodegeneration in both the caudate and putamen follows a caudo-rostral progression, with a preferential dorsal to ventral gradient. Thus, at end stage cell loss is maximal in the tail of the caudate nucleus, less severe in the body, and least in the head of the caudate, although in severe cases neuronal populations are devastated throughout the neostriatum (100). Fibrillary astrogliosis follows the path of cell loss in striatum, however reac-

Figure 1. Immunocytochemical localization of lipofuscin in cortical pyramidal neurons and striatal neurons, but lack of colocalization with NADPH-diaphorase staining, in HD brain. Plates are frontal cortex (Brodmann area 8) and caudate nucleus sections single- and double-stained for SMI-32 (neuro-filament), acid phosphatase (lipofuscin), and NADPH-diaphorase (NADPH-d). **A**) Low power view of the cortex immunocytochemically stained with SMI-32 antisera. Note the pyramidal distribution of SMI-32 labeling, predominantly in laminae 2, 3 and 5. **B**) Low power view of the same cortical region as in A, staining acid phosphatase activity (lipofuscin). The staining distribution pattern is equivalent to SMI-32 labeling. **C**) High power photomicrograph of a cortical section double-stained for SMI-32 and acid phosphatase activity, demonstrating colocalization of SMI-32 labeling with punctate acid phosphatase (lipofuscin) within the same pyramidal neuron. **D**) Photomicrograph of a neocortex section double-stained for acid phosphatase and NADPH-diaphorase. There is little or no phosphatase activity within the NADPH-diaphorase positive neuron. **E**) Photomicrograph of a striatal section double-stained for acid phosphatase and NADPH-diaphorase. As in D, there is little or no colocalization of the endproducts. However, **F**) acid phophatase can be identified in a small population of NADPH-d neurons within the striatum, although it is less pronounced than in surrounding non-NADPH-d neurons.

tive gliosis does not accompany the chronic cell loss seen elsewhere in the brain, and no inflammatory response is involved (73).

On the basis of the progressive involvement of striatal regions, Vonsattel developed guidelines for grading HD patients based on gross and microscopic measures of neuropathological severity, determined in three standardized coronal brain sections including the striatum (99). Grades range from 0 to 4 with increasing severity and extent of striatal involvement. Briefly, Grade 0 cases are generally indistinguishable from normal brains on gross examination, but exhibit 30-40% neuronal loss in the head of the caudate, with no visible signs of reactive gliosis. In contrast, in Grade 4 the striatum is severely atrophic with loss of more than 95% of neurons and markedly increased oligodendrocytic density. Cell loss in the nucleus accumbens is evident in approximately 50% of Grade 4 cases. The majority of HD cases (approximately 80%) are Grades 3 or 4 at time of death (100). The grade of striatal pathology appears to correlate closely with the involvement of other cerebral regions. Non-striatal regions are largely unaffected in grades 1 and 2, whilst atrophy and neuronal loss are evident in the GP, cortex, and to a lesser extent thalamus, subthalamic nucleus, substantia nigra, white matter and cerebellum in Grade 3 and 4 cases (17, 92, 100). Cerebellar atrophy is particularly prevalent in cases of juvenile onset HD.

Surviving neostriatal neurons generally appear morphologically normal, but may be reduced in size and contain elevated levels of the oxidative damage marker lipofuscin. However Vonsattel has described a sub-population of neurons scattered largely between the zones of atrophic and healthy cells, referred to as "neostriatal dark neurons" (NDN) because of their relatively intense staining with Luxol-fast-blue and haematoxylin and eosin. These cells have characteristically scalloped membranes, granulation of the cytoplasm and condensation of nuclear chromatin, and some can be labeled by TdT-mediated dUTP-biotin nick end-labelling (TUNEL), suggesting that they may be undergoing apoptosis (100).

Clinical Correlates of Neuronal Loss. *Striatal Neuronal Populations in HD.* The striatal cell type most susceptible to degeneration in HD is the medium spiny projection neuron (81, 82, 99). Spiny neurons constitute 80% of striatal neurons, and are the principal input and output neurons of the striatum. Spiny neurons may be classified as small-, medium- or large-sized, but all contain the inhibitory neurotransmitter γ-aminobutyric acid

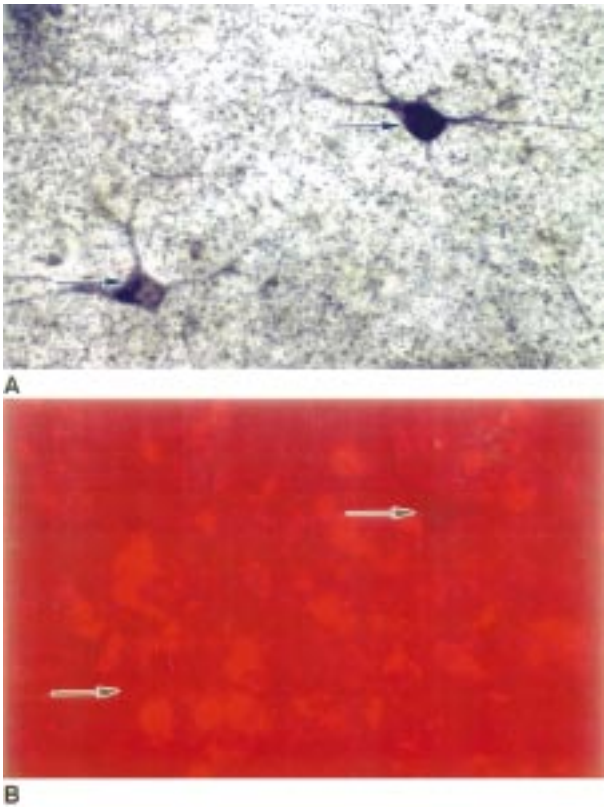


Figure 2. Photomicrographs of a double-stained tissue section from human HD caudate nucleus using NADPH-diaphorase enzyme histochemistry (**A**) and immunofluorescence for acid phosphatase activity (lipofuscin) (**B**). Two NADPH-diaphorase neurons are identified by arrows in A. The same area of the section was photographed in B for acid phosphatase activity. Bright red identifies lipofuscin within neurons. There is little or no colocalization of lipofuscin within NADPH-diaphorase neurons.

(GABA). Subsets of spiny neurons also contain enkephalin (ENK), substance-P (SP), dynorphin or calbindin. The other major class of striatal neuron are aspiny interneurons. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), neuropeptide Y (NPY), somatostatin (SS) and nitric oxide synthase (NOS) typically colocalize in medium-sized aspiny neurons, and some contain cholecystokinin (CCK) or the calcium binding protein parvalbumin. The large aspiny neurons contain acetylcholine. In HD striatum, spiny projection neurons containing SP or ENK are involved earliest and most severely, whereas aspiny interneurons and the larger cholinergic interneurons are relatively spared (9, 40). There is also some hierarchy in vulnerability of different spiny neuron subsets, ENK-positive neurons projecting to the external segment of the globus pallidus (GPe) degenerating prior to SP-containing neu-

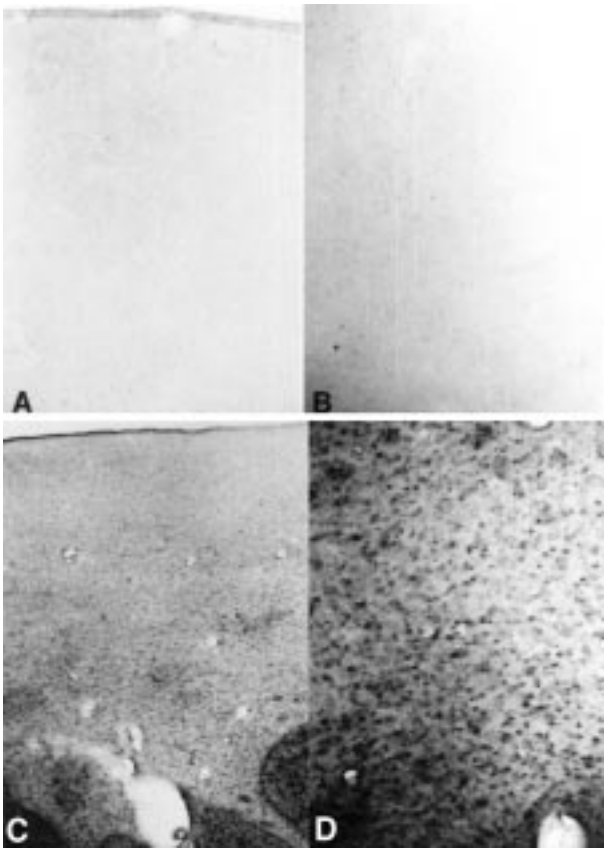


Figure 3. In situ end labeling (ISEL) detection of DNA fragmentation in normal control (**A**, **B**) and HD caudate (**C**, **D**), at low and higher power, respectively. ISEL staining is significantly greater in HD than in control striatal neurons. Note that ISEL is distributed predominantly within the cytoplasm with little or no labeling within the nucleus, implying more severe involvement of mitochondrial than nuclear DNA.

rons projecting to the internal segment (GPi) (81, 82, 84). Golgi staining shows that spiny neurons are also susceptible to morphological changes in HD, including recurving of the dendrites, altered shape and size of the spines, and increased density of spines.

Motor Dysfunction in HD. The clinical symptoms of HD reflect the pattern and extent of neuronal loss within different components of the basal ganglia-thalamocortical circuit. The neostriatum (caudate nucleus and putamen) receives excitatory glutamatergic inputs from the entire neocortex, the first step in the anatomical loop responsible for the initiation and execution of movement. Processed signals are transmitted via basal ganglia output nuclei (the internal segment of the globus pallidus, GPi; the substantia nigra pars reticulata, SNr; and ventral pallidum) to the thalamus, which in turn sends excitatory projections to areas of the frontal cortex associated with motor planning and execution (2, 4, 47). The GABAergic basal ganglia output projections to the thalamus maintain a tonic inhibition of their target nuclei, which is modulated by two opposing pathways (direct and indirect) which integrate the input and output compartments within the basal ganglia. It is an imbalance in the relative contributions of these two regulatory pathways which triggers, and dictates the nature of, the motor dysfunction in HD.

In the direct (monosynaptic) pathway, striatal efferents containing GABA and SP (GABA/SP) project directly to the internal segment of the globus pallidus (GPi). Activation of this pathway results in disinhibition of thalamic activity. In contrast, the indirect pathway consists of a polysynaptic projection between the striatum and GPi. In the first step of the indirect pathway, striatal efferents containing GABA and enkephalin (GABA/ENK) project to the external segment of the globus pallidus (GPe) which then sends purely GABAergic projections to the subthalamic nucleus. The efferent projection to the basal ganglia output nuclei (SNr and GPi) from the subthalamic nucleus is excitatory, most likely glutamatergic. The GPe projection generally exerts a tonic inhibition on the subthalamic nucleus, and activation of the GABA/ENK striatal efferents tends to suppress the activation of GPe neurons, resulting in disinhibiton of the subthalamic nucleus and hence an increase in the excitatory innervation of the basal ganglia output nuclei. This leads to an increased inhibitory input to the target thalamic nuclei. Consequently the thalamic innervation of the cortex is differentially modulated depending on the basal ganglia pathway activated (2, 4). Disruption of these pathways in HD leads to motor dysfunction. In HD there is preferential loss of the GABA/ENK neurons comprising the indirect pathway. The resulting "disinhibition" of the thalamus is manifest by the development of involuntary choreic movements. In contrast, it is proposed that the rigid akinetic state seen in some HD patients results from the additional loss of striatal GABA/SP efferents projecting directly to the GPi (1). There is also recent evidence that the dyskinesia seen in HD patients is influenced by imbalances between neuronal activities within the basal ganglia internal and external pallidal segments, as well as between segments (68).

Neurotransmitter Factors Influencing Cell Vulnerability in the HD Striatum. Several factors including cell localization, afferent and efferent projections, and neurochemical content appear to influence cell fate in HD. It has been proposed that the variable rates of cell death within the striatum and the selective neuronal vul-

nerability in HD may reflect the neuronal distribution of glutamate receptor subtypes. The spiny neurons most vulnerable to degeneration contain mainly N-methyl-D-aspartate (NMDA) glutamate receptor subtypes (predominantly NMDAR-1 and NMDAR-2B) which are implicated as mediators of excitotoxic cell death in numerous pathological situations (27). Furthermore, an early and preferential loss of NMDA receptors in HD has been demonstrated (103). In contrast, the relatively preserved aspiny interneurons are rich in GluR-1 AMPA ionotropic glutamate receptor subtypes. Striatal spiny neurons also contain high densities of mGlu5 (group 1) and mGlu3 (group II) metabotropic glutamate receptor subtypes, which modulate excitatory synaptic transmission via activation of G-proteins in cell membranes. Group I mGluR subtypes have been proposed to be involved in neurotoxic processes, whereas group II subtypes are protective. Dopaminergic (DA) transmission also appears to influence cell vulnerability in the striatum. Striatal spiny neurons contain D1 receptors in the direct pathway, and D2 receptors in the indirect pathway. D1 and especially D2 receptors are markedly reduced in asymptomatic HD mutation carriers, suggesting that loss of DA innnervation contributes to the pathophysiology of HD (101).

NADPH-d-containing aspiny interneurons are relatively resistant to degeneration in HD. This has been postulated to reflect the type of glutamatergic receptors they express, and to be associated with their co-localization with NOS. NOS-containing neurons in the striatum are historically resistant to acute excitotoxic insults putatively mediated by nitric oxide (NO), including ischemic insults, leading to the proposition that the capacity to generate NO confers some degree of protection on this cell type (57, 98). A recent report suggests that this resistance to NO toxicity may be related to antioxidant properties of these cells, since cultured NO-resistant neurons were found to contain elevated levels of the mitochondrial superoxide radical scavenging enzyme manganese superoxide dismutase (MnSOD) (45). Furthermore, presence of MnSOD was found to be critical for the resistance of these nNOS-positive cultured neurons to NO- and NMDA-mediated toxicity. The involvement of excitotoxic processes is inferred by observations that intrastriatal injections of the endogenous NMDA receptor agonist quinolinic acid induce preferential loss of medium spiny neurons but spare NADPH-d neurons, whilst injection of the non-NMDA receptor agonists kainate or quisqualate result in loss of both spiny and NADPH-d positive aspiny neurons (8, 57). In contrast, s-4-carboxy-3-hydroxyphenylglycine (a group II mGluR agonist and a group I mGluR antagonist) is protective against quinolinic acid lesions (75). However, the extreme striatal atrophy and neuronal loss seen in Grade 4 HD patients suggests that ultimately all cell types are susceptible to cell death in HD.

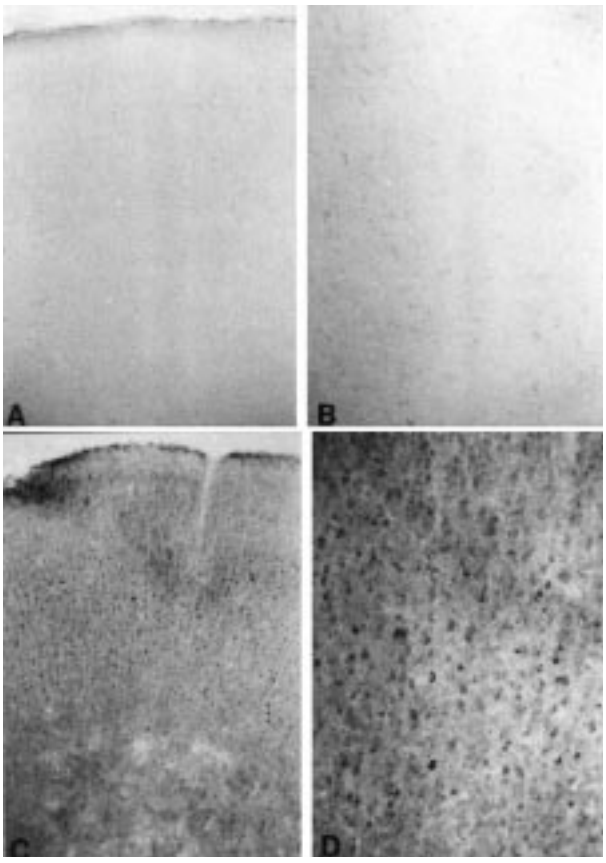
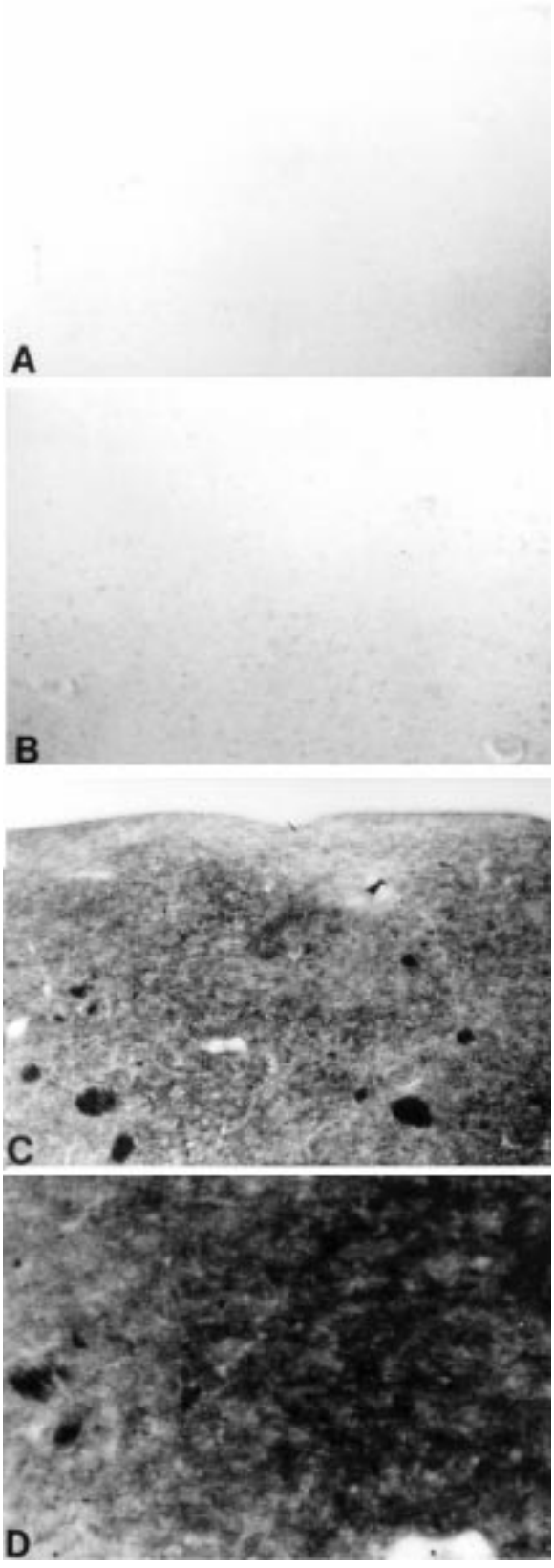


Figure 4. In situ end labeling (ISEL) detection of DNA fragmentation in normal control (**A**, **B**) and HD cortex (**C**, **D**), at low and higher power, respectively. The amount of label is markedly increased in HD cortex, relative to age-matched control levels.

Mutant Huntingtin Protein and the HD Brain. The genetic defect in HD is an expansion of an unstable CAG repeat encoding polyglutamines at the 5' end of a gene on chromosome 4, termed "interesting transcript 15" (IT15) (97). cDNA analysis reveals a predicted 348 kD gene product containing 3144 amino acids, termed "huntingtin" protein. In unaffected individuals the IT15 trinucleotide repeat typically contains 11-34 CAGs. Expansion to 35-39 CAG repeats in one or both alleles confers the likelihood of developing HD, whilst individuals with greater than 39 CAG repeats in either allele will develop the disease. The trinucleotide repeat is polymorphic and undergoes alterations during meiosis,



generally fluctuating by ± 1-5 repeats per transmission, although larger increases can occur following paternal transmission (83). At present the physiological functions of both normal and mutant huntingtin are unknown. However it is known that several features of the HD phenotype are influenced by CAG repeat length in the mutant gene. Most individuals develop the first symptoms of HD in adulthood, although age of onset of the disease is inversely correlated with size of the CAG repeat expansion, and a small proportion of cases have a juvenile onset associated with large CAG repeat lengths (5, 36). CAG repeat length has also been correlated with neuropathological severity, although this observation is controversial since grade of disease at time of death is dependent on a number of factors also influenced by repeat length, including age of onset and disease duration (43).

The elucidation of a pathogenic function for mutant huntingtin is currently the subject of intense research activity, but to date little is known about its potential roles in cell death processes, and distribution studies give little insight into the regional selectivity of cell loss in the disease. Studies in huntingtin "knock-out" mice suggest that the mutation results in the gain of a novel function, since murine *HD* homologue null mice die *in utero*, whilst heterozygous knock-out mice show little or no pathology (37). In addition, mice expressing abnormally low levels of murine huntingtin exhibit developmental abnormalities (102). Observations that antibodies to expanded polyglutamine stretches can discriminate between normal and mutant forms of huntingtin imply that the gene defect causes a conformational change in the protein (96). Findings that huntingtin protein is widely expressed throughout the body, showing no apparent selectivity for cerebral regions targeted by the disease process, suggest that another property of basal ganglia neurons confers vulnerability to degeneration in HD (62, 90). However, immunohistochemical studies have recently revealed a heterogeneous distribution of huntingtin immunoreactivity within the striatum which may underly cell susceptibility (39). Ferrante and colleagues (39) report huntingtin immunoreactivity located primarily within the matrix compartment, whereas little or no immunoreactivity was seen in the patch compartment. Double labeling techniques

Figure 5. 3-Nitrotyrosine (3-NT) immunoreactivity, a marker for protein nitration, is increased in HD caudate. Relative to levels of immunoreactivity in normal caudate nucleus (**A**, **B**), there is a marked increase in immunohistochemical expression of 3-NT in HD striatum, predominantly localized to neuronal and neuropil elements.

revealed higher levels of huntingtin expression in medium spiny neurons and colocalization with calbindin, in contrast to little or no colocalization between huntingtin and NADPH-d or NOS neurons.

In neurons, huntingtin protein has a cytoplasmic distribution in perikarya, axons, dendrites and some nerve terminals. Potential roles in intracellular trafficking and synaptic function have been proposed on the basis of subcellular fractionation studies which indicated an association of huntingtin with synaptic vesicles and microtubules (32, 50). Huntingtin protein has also recently been identified in neuronal nuclei. N-terminal fragments of huntingtin have been found in ubiquinated protein aggregates deposited in neuronal nuclei (neuronal intranuclear inclusions, NII) and in dystrophic neurites (cytoplasmic inclusions, CI). These protein aggregates have been identified in both HD brain, and in the brains of transgenic mice expressing a fragment of human mutant huntingtin (30, 33). The mechanism of inclusion formation is unclear at present, but Perutz and colleagues (79) have suggested that the expanded polyglutamine stretches in mutant huntingtin facilitate the formation of β-pleated sheets held together by hydrogen bonds between amide groups. The CAG repeat length appears to be critical for aggregate formation, and Scherzinger and colleagues (86) demonstrated the formation of insoluble high molecular weight protein aggregates following proteolytic cleavage of a GST-huntingtin fusion protein containing 51 glutamine residues. However no protein aggregation occurred when polyglutamine repeat stretches were reduced to 20 or 30. Similar intranuclear inclusions have also been reported in other CAG repeat disorders including spinocerebellar ataxia type 3 (78). It is yet to be determined whether these huntingtin protein aggregates are directly involved in processes of cellular dysfunction, or whether deposition is secondary to another pathogenetic process.

Functional Alterations in HD Brain

Energy Metabolism is Selectively Disrupted in Brain Regions Targeted in HD. *Human Studies.* Despite identification of the HD mutation gene product, it is still not known how the gene defect results in selective cell death. One hypothesis is that the gain of function associated with expanded polyglutamine repeats leads either directly or indirectly to a defect in mitochondrial energy metabolism. As discussed later, mitochondrial defects may result from oxidative damage to cellular elements, or alternatively may induce oxidative stress via increased free radical production.

Mitochondrial abnormalities in HD were first identified in ultrastructural studies of cortical biopsies from juvenile and adult onset HD cases (44). A metabolic defect in HD is also implicated by observations of insidious weight loss in HD patients despite a sustained caloric intake (77). The bulk of evidence comes from positron emission tomography (PET) studies and biochemical studies in post-mortem brain which show selective metabolic defects in brain regions targeted by the disease.

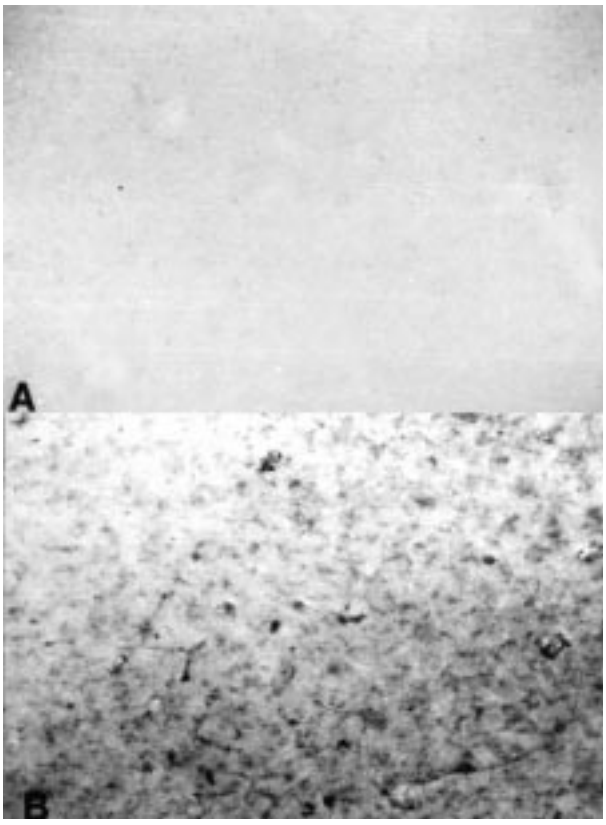
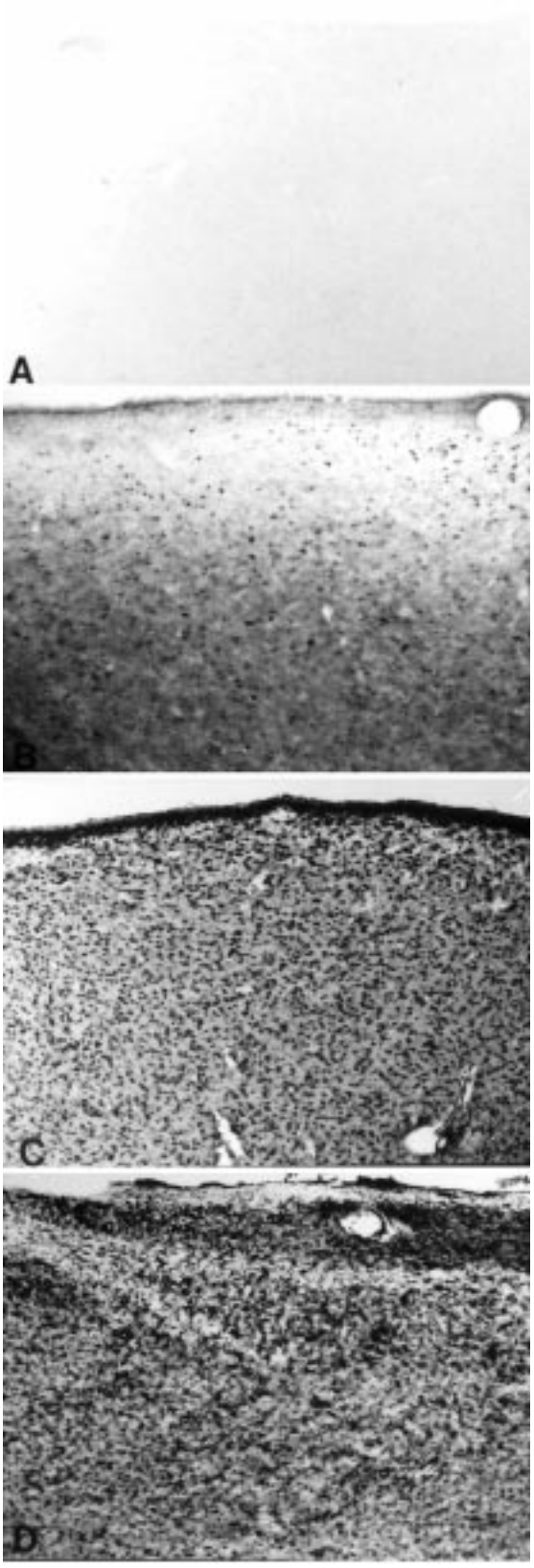


Figure 6. Malondialdehyde immunoreactivity, detecting lipid peroxidation, is increased in HD caudate nucleus (**B**), relative to levels in normal control caudate nucleus (**A**), and is evident in neuronal, astrocytic and vascular tissue elements.

Glucose metabolism is markedly reduced in the basal ganglia and cerebral cortex of symptomatic HD patients (23, 60, 61, 70). Moreover, findings in PET studies that rates of striatal glucose utilization are reduced prior to the bulk of tissue loss in HD, and in asymptomatic subjects at risk of developing the disease provide compelling evidence that energetic defects play a causative role in the disease process (23, 60, 70). Cortical hypometabolism is also seen in patients suffering psychological disturbances and mood changes, before the onset of motor symptoms (61). Nuclear magnetic resonance (NMR) imaging studies also demonstrate metabolic abnormalities in HD brain, suggestive of enhanced



glycolytic activity to compensate for impaired oxidative phophorylation (55, 58). Elevated lactate concentrations were found in the occipital cortex and basal ganglia of symptomatic HD patients, but were not altered in asymptomatic or at risk patients (55, 56). The degree of increased lactate production correlated well with the duration of the disease, implying that normal energy metabolism is progressively impaired by the disease process. Interestingly, increased lactate production in HD brain can be ameliorated by treatment with the metabolic co-factor and antioxidant coenzyme Q_{10} (58).

Biochemical studies in HD post-mortem tissue show selective dysfunction of components of the oxidative phosphorylation pathway and Krebs' cycle in brain regions targeted in the disorder. Activities of succinate dehydrogenase 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 (22, 25, 49). Brennan and colleagues (18) have also reported decreased cytochrome aa_3 levels in HD caudate, whereas cytochome b and cc1 levels do not change in the disease. Reduced pyruvate dehydrogenase activity has been reported in the basal ganglia and hippocampus in HD, while polarographic studies show decreased oxygen consumption in HD striatum, relative to levels in age-matched controls (25).

Animal Models of HD: Mitochondrial Toxins. A role for mitochondrial energy metabolism dysfunction in the pathogenesis of neuronal degeneration in HD is further supported by observations, in both humans and in experimental animals, that the basal ganglia neurons are particularly vulnerable to mitochondrial toxins such as the complex II inhibitors 3-nitropropionic acid (3-NP) and malonate; aminooxyacetic acid (AOAA) and MPTP (complex I); potassium cyanide and sodium azide (complex IV) (7, 10, 13, 20, 48, 65). Ingestion of 3-NP, an irreversible inhibitor of succinate dehydrogenase (complex II), produces selective basal ganglia lesions and delayed dystonia in humans (65). Systemic administration of 3-NP to both rats and primates produces age-dependent striatal lesions which are strikingly similar to those seen in HD (20, 21). In primates, chronic 3-NP

Figure 7. 8-Hydroxy-deoxyguanosine (8-OHdG) expression, a marker of DNA oxidation, is significantly increased in HD striatum, and levels of expression in cells and neuropil increase with severity of the disease. In comparison to immunoreactivity in normal caudate nucleus (**A**), 8-OHdG expression is moderately increased in low garde HD (**B**, Vonsattel Grade 1), and to a greater extent in moderate (**C**, Grade 2) and severe (**D**, Grade 4) HD. Less cellular staining in **D** may reflect greater neuronal loss.

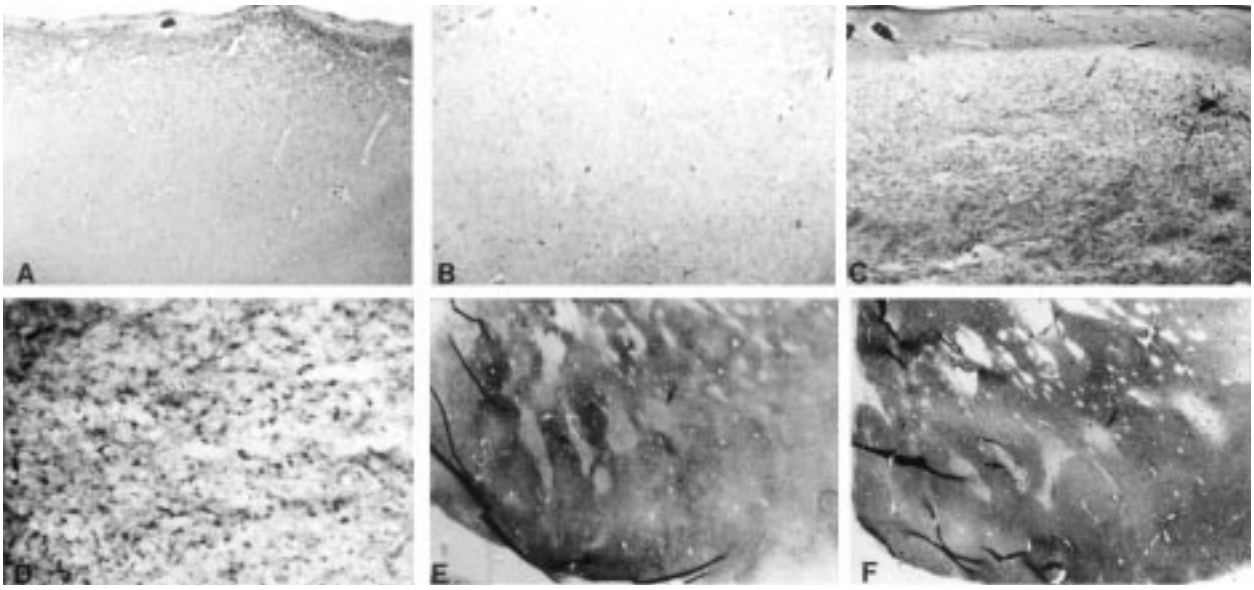


Figure 8. Heme oxygenase 1 (HO1), a marker for inducible oxidative stress, is increased in HD striatum. Relative to levels in normal caudate nucleus (**A**, **B**), there is a marked increase in HO1 immunoreactivity in HD caudate (**C**, **D**) associated with cellular and vascular tissue elements. Topographic heterogeneity of of HO1 immunoreactivity was observed within the HD caudate. Areas of greatest HO1 immunoreactivity (**E**) conformed to the matrix compartment , as shown in a contiguous striatal section stained for calbindin-Dk28 immunoreactivity (**F**). Arrows identify comparable patches of low activity.

administration produces selective striatal lesions which spare NADPH-d neurons, and induce proliferative changes in the dendrites of spiny neurons. Animals also show both spontaneous and apomorphine-inducible movement disorders resembling HD (21). 3-NP basal ganglia lesions in rats are associated with elevated lactate levels, similar to the increased lactate production seen in HD patients (20). 3-NP lesion formation in rats can be blocked by removal of glutamatergic excitatory striatal inputs by decortication, by glutamate release inhibitors, and by glutmate receptor antagonists, suggesting that 3-NP toxicity is mediated by secondary excitotoxic mechanisms (7, 89).

Increased Energy Metabolism is Neuroprotective in HD and Animal Models of HD. Further indirect evidence that energetic defects contribute to neurodegenerative processes in HD is provided by evidence that agents which enhance energy production in the brain exert neuroprotective effects. Preliminary studies in rodent mitochondrial toxin models, and NMR measurements of lactate production in man, suggest that coenzyme Q_{10} and creatine are neuroprotective, putatively via enhancing cerebral energy metabolism (58, 69). A number of agents which may improve mitochondrial function have been suggested as potential therapeutic strategies. These include vitamins that are coenzymes of respiratory enzymes, such as thiamine, riboflavin or biotin, or agents which serve as supplementary electron donors and acceptors, such as vitamins C, K_3 and coenzyme Q_{10} (CoQ_{10}). Coenzyme Q_{10} also has potent antioxidant effects. To date CoQ_{10} has been investigated most thoroughly, and oral administration improves symptoms in some mitochondrial-associated disorders including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) and Kearns-Sayre syndrome patients (reducing CSF and serum lactate and pyruvate levels, and enhancing mitochondrial enzyme activities in platelets) (19, 52). We recently showed that oral administration of CoQ_{10} over 1-2 months significantly ameliorated elevated lactate levels seen in the cortex of HD patients, an effect which was reversible on withdrawal of CoQ_{10} (58). Furthermore, CoQ_{10} attenuates neurotoxicity induced by the mitochondrial toxins MPTP and malonate in animal models (13, 89).

An alternative strategy is to increase brain energy stores of the high energy compound phosphocreatine (PCr) by creatine administration. Creatine is phosphorylated by mitochondrial creatine kinase. PCr then essentially shuttles energy in the form of a high-energy phosphate bond from the mitochondria to cell regions where it is required. ATP is regenerated in the cytoplasm where it is used in many different functions including maintenance of the membrane potential by the Na^+/K^+-ATPase pump, and for Ca^{2+} buffering by Ca^{2+}-ATPase (51). It is

hypothesized that PCr may be neuroprotective during periods of energetic compromise by providing an alternative energy source to ATP. This hypothesis is supported by observations that PCr levels are reduced prior to any ATP loss during depletion of energy stores in ischemic conditions. We recently showed that oral creatine administration in rats attenuates neurotoxicity induced by the succinate dehydrogenase inhibitor 3-NP (69). In addition, increases in cerebral lactate levels and decreases in levels of high energy phosphate compounds seen in the striata of 3-NP treated rats were attenuated by pre-treatment with creatine.

Evidence of Oxidative Damage In HD. *Human Studies.* Abnormal increases in levels of a number of markers for oxidative damage have been reported in HD brain. These include increased incidence of DNA strand breaks, exacerbated lipofuscin accumulation, and increased immunohistochemical staining of oxidative damage products in HD striatum and cortex (see Figures 1-8) (41, 44, 94).

A number of studies indicate increased peroxidative damage in HD. These include evidence that HD skin fibroblasts show increased vulnerability to glutamate toxicity which can be attenuated by antioxidant treatment (71), and findings of enhanced lipofuscin deposition in HD brain (94). Lipofuscin is a fluorophore produced by the reaction of amino compounds with secondary aldehydic products of oxidative free radical-induced oxidation of macromolecules, in particular lipid peroxidation. It accumulates in lysosomes in post-mitotic cells such as neurons and cardiac myocytes, and is commonly known as "age pigment" since its accumulation increases with age (95). The rate and extent of lipofuscin deposition in cells also increases under conditions of increased oxidative stress and metabolic rate (74, 91). As shown in Figures 1 and 2, both striatal and cortical neurons in HD brain contain higher levels of lipofuscin than age-matched controls. In addition, the extent of lipofuscin accumulation increases with the neuropathological severity of the disease. These results suggest increased lipid peroxidative damage in affected brain regions in HD.

Free radical-induced oxidative damage to DNA can induce DNA strand breaks (34). A number of studies show evidence of increased DNA strand breaks in HD striatal neurons, and have suggested the involvement of both apoptotic and necrotic mechanisms of cell death (26, 80). Moreover, a recent study demonstrates that the degree of DNA fragmentation in HD striatum is positively correlated with the length of the polyglutamine expansion in huntingtin (26). Using *in situ* end labelling techniques (ISEL), we found marked increases in DNA fragmentation in HD striatal and cortical neurons, relative to levels in the corresponding regions of age-matched controls (Figures 3 and 4). Furthermore, it appears that mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA (nDNA) to fragmentation, since little or no ISEL labeling was detected within cell nuclei.

Oxidative damage can also induce excessive oxidation of DNA bases, such as deoxyguanosine (to produce 8-hydroxy-deoxyguanosine; 8-OHdG) (42). We found significant increases in levels of 8-OHdG in nDNA in the caudate of Grade 4 HD cases, using HPLC detection methods in post-mortem tissue (22). No alterations in nDNA 8-OHdG content were seen in brain regions affected to a lesser extent in HD, including frontal and parietal cortices and cerebellum, in the same subject population.

Using immunohistochemical markers to examine oxidative damage to a number of cellular macromolecules, we found abnormal elevations in oxidative damage to lipid, protein and DNA in HD cortex and striatum (Figures 5-8; 41). In control brains, we found that immunoreactive staining for 3-nitrotyrosine (3-NT; a marker for peroxynitrite-mediated protein nitration), malondialdehyde (MDA; marker for oxidative damage to lipids), 8-OHdG (DNA oxidative damage product) and heme oxygenase (HO; formed during oxidative stress) was weak, evident only in scattered cortical and striatal neurons, with enhanced staining in the striatal matrix zone. The extent and intensity of immunoreactivity were enhanced in HD brain, and patterns of staining seen in cases of different grades mirrored the pattern of progressive cell loss throughout the brain. In low grade HD cases, 3-NT, MDA, 8-OHdG and HO all showed greater immunoreactivity in dorsal versus caudal striatum, and the extent of immunoreactivity increased through the striatum with increasing grade of disease. In grades 3 and 4 striatal immunoreactivity was reduced, consistent with the severe striatal cell loss at end stage

Animal Studies. Oxidative damage is also implicated in the pathogenesis of striatal lesion formation induced by the mitochondrial toxins malonate and 3-NP, which inhibit succinate dehydrogenase and produce striatal lesions resembling HD (88, 89). Intrastriatal injection of either agent in rats increased the rate of hydroxyl free radical production in the striatum, as detected by microdialysis measurement of 2,3- and 2,5-dihydroxybenzoic acid levels, generated by the reaction of hydroxyl radicals with salicylate. Increased 8-OHdG levels in stria-

tum were also detected following systemic 3-NP administration, and elevated 3-nitrotyrosine concentrations were seen after either systemic 3-NP administration, or intrastriatal malonate injection. Lesion volumes and associated increases in oxidative damage markers induced by 3-NP were markedly attenuated in mice overexpressing the superoxide free radical scavenger Cu/Zn superoxide dismutase (Cu/Zn-SOD), suggesting the involvement of oxidative free radical damage in lesion formation (14). Furthermore, malonate and 3-NP striatal lesions were attenuated by free radical spin traps and nitric oxide synthase (NOS) inhibitors (89). Inhibition of nitric oxide (NO) generation in mice lacking the gene for the neuronal isoform of NOS (nNOS), also resulted in reduced volume of malonate lesions. Results support the involvement of nitric oxide-mediated oxidative damage in cell death processes following energetic disruption in these models (89).

Putative Mechanisms of Cell Death in HD: Mitochondrial Damage, Oxidants and Excitotoxicity.

Mitochondrial Dysfunction and Excitotoxicity. What are the common pathways linking the huntingtin mutation with bioenergetic defects, oxidative damage and cell loss in HD? The definitive answer is currently unknown, but one hypothesis is that bioenergetic defects could lead to neuronal death via so-called secondary excitotoxicity (3, 6). Energetic defects might occur as a primary event in HD, or as a consequence of oxidative damage to cellular elements. Reduced ATP production due to impaired mitochondrial energy metabolism can result in partial cell depolarization, by making neurons more vulnerable to endogenous levels of glutamate (15, 28). The concomitant increase in Ca^{2+} influx into neurons may trigger further free radical production, exacerbating damage to cellular elements. This hypothesis is supported by findings that normally ambient levels of excitatory amino acids become toxic in the presence of oxidative phosphorylation inhibitors, sodium-potassium pump inhibitors, or potassium-induced partial cell membrane depolarization (76, 104). Further, excitatory amino acid antagonists such as MK-801 can ameliorate cerebral lesions induced by mitochondrial toxins including AOAA, malonate, 3-NP and 3-acetylpyridine, 3-AP (10, 48, 65, 87). Excitotoxic mechanisms have been implicated in the mechanism of cell death in HD largely on the basis of observations of NMDA glutamate receptor distribution within striatal cell populations, and findings that excitotoxic striatal lesions in animal models closely resemble those seen in HD brain (6, 8).

Impaired activity of components of the energy metabolism pathways including pyruvate dehydrogenase and succinate dehydrogenase have been reported in HD, as discussed previously. Direct impairment of the mitochondrial oxidative phosphorylation pathway, or reduced substrate trafficking into this pathway as a result of disruption of glycolysis or the Krebs cycle, will result in reduced ATP production by mitochondria. The bulk of ATP production within cells takes place within the mitochondrial electron transport chain. It is comprised of five protein complexes: NADH ubiquinone oxidoreductase (complex I), succinate ubiquinol oxidoreductase (II), ubiquinol cytochrome c oxidoreductase (III), and cytochrome c oxidase (IV) which act in series to oxidize NADH and $FADH_2$, generated in the mitochondrial matrix by the Krebs cycle, via electron transfer, ultimately to oxygen. Electron movement involves pumping of protons across the inner mitochondrial membrane, producing an electrochemical gradient which complex V (ATP synthase) utilizes as an energy source for the high-energy bonds in ATP. Optimal metabolic activity is dependent on the efficiency of electron movement along the electron transport chain and its coupling to oxidative phosphorylation, and processes which disrupt electron transfer may potentially impair mitochondrial energy metabolism.

Reduced ATP production can result in cell death via disruption of energy-dependent processes (6). ATP is essential to fuel ionic pumps which generate and maintain ionic and voltage gradients across neuronal membranes, including Na^+/K^+-ATPase pumps which control the restoration of the resting membrane potential after depolarization, and ATPases which regulate intracellular levels of Ca^{2+}. Impaired Na^+/K^+-ATPase pump activity will inhibit membrane repolarization, resulting in prolonged or inappropriate opening of voltage-dependent ion channels. If severe enough, this partial membrane depolarization may facilitate activation of NMDA receptors by endogenous levels of glutamate, by alleviating the voltage-dependent Mg^{2+} blockade of NMDA receptor channels. The resultant excessive inward flux of Na^+ and Ca^{2+} ions can set in motion neurotoxic cascades. Ca^{2+} influx into neurons is implicated as a trigger for increased free radical production via NO-mediated mechanisms, and associated oxidative damage to cellular elements including proteases, lipases and endonucleases, ultimately leading to cell death. The cascade may become self-perpetuating, high cytosolic Ca^{2+} levels inducing Ca^{2+} uptake by mitochondria, leading to irreversible mitochondrial damage which is exacerbated by the actions of Ca^{2+}-activated phospholipases. Increased

intracellular Na+ levels will decrease the activity of the Na^+/Ca^{2+} antiport system, which would normally extrude Ca^{2+} from the cell. In addition, reduced ATP levels will impair ATP-dependent extrusion and storage of Ca^{2+}, further increasing intracellular concentrations of free Ca^{2+}.

Oxidative Damage and Energetic Dysfunction. It is presently unclear whether mitochondrial dysfunction is a direct consequence of the huntingtin mutation in HD, or occurs as a secondary event. However, recent evidence suggests that mitochondrial energy metabolism dysfunction may result from excessive oxidative damage to DNA or other neuronal macromolecules, as a consequence of increased free radical and oxidant generation. Free radicals including superoxide ($O_2^{\bullet}$-) and hydroxyl radicals ($HO^{\bullet}$-) are constantly produced as by-products of aerobic metabolism, but production increases under circumstances of electron transport chain inhibition or molecular defects (35, 38). Elevated Ca^{2+} influx induced by excitotoxic processes leads to sequestering of Ca^{2+} in mitochondria, which in turn increases free radical generation by the mitochondria. Increased generation of hydroxyl and carbon-centered radicals by mitochondria in response to Ca^{2+} concentrations similar to those induced by neuronal exposure to excitotoxins has been demonstrated *in vitro* (38). Redox sensitive dyes, including dihydrorhodamine, have also shown glutamate-induced increases in mitochondrial Ca^{2+} content and free radical generation *in vitro* (35). Incresed nitric oxide (NO) production in response to elevated Ca^{2+}/calmodulin-mediated activation of NOS, may also result in increased peroxynitrite ($ONOO^-$) formation. $ONOO^-$, produced by the reaction of NO with superoxide radical, may then react with Cu/Zn-SOD to form nitronium ion, which nitrates tyrosine residues in proteins (53). An alternative pathway for NO/$ONOO^-$-mediated toxicity is via peroxidative DNA damage leading to activation of poly(ADP-ribose) synthetase (PARP). PARP is a nuclear enzyme invoved in DNA repair, but excessive PARP activation can exhaust cellular energy supplies, inducing cell death cascades due to energetic dysfunction (105).

Free radicals can induce oxidative damage to cell macromolecules including DNA, proteins and lipids by a number of different mechanisms, including DNA strand breaks or formation of DNA adducts such as 8-OHdG, protein carbonylation, or by lipid peroxidation (42, 67, 93). Potential functional consequences include perturbations of DNA transcription and translation, protein synthesis, enzyme activities and membrane fluidity. Mitochondria are thought to be particularly vulnerable to oxidative injury since most intracellular free radicals are generated by the mitochondrial electron transport chain. MtDNA is extremely susceptible due to its localization in the mitochondrial matrix, lack of protective histones, and limited repair mechanisms (64). Thus any increase in free radical production, or impaired activity of a regulatory enzyme such as Cu/Zn-SOD or glutathione peroxidase, could reduce the functional capacity of the oxidative phosphorylation pathway. In addition, the slow, progressive nature of neuronal injury in chronic neurodegenerative disorders may be explained by cycling of free radicals and mitochondrial dysfunction. Further evidence supporting a role for oxidative damage in HD is that the energetic defects seen in HD brain are similar to those induced in cell culture by peroxynitrite, which preferentially inhibits complex II-III and (to a lesser extent) complex IV activity in the electron transport chain (16).

Pathological Interactions of Mutant Huntingtin Protein? A number of possible cellular interactions of mutant huntingtin have been proposed. These include suggestions that expanded glutamine repeats may allow protein-protein interactions, or that polyglutamines may be a substrate for transglutaminase (29, 79). The principal candidate protein interactors are huntingtin-associated protein-1 (HAP-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (24, 63). HAP-1 is found only in brain but does not show a preferential striatal distribution. GAPDH is a critical glycolytic enzyme, leading to suggestions that an interaction with huntingtin may result in impaired metabolic function. The potential for huntingtin to deleteriously affect GAPDH function is supported by a recent report that both GAPDH and α-ketoglutarate dehydrogenase are inactivated by fusion proteins containing polyglutamine stretches of pathological length, in reactions catalyzed by transglutaminase (29). However, the glycolytic function of GAPDH appears to remain intact in HD, as a recent study failed to detect any differences in levels of cytosolic GAPDH activity between HD grade 4 and age-matched control brain tissue (22).

There is a great deal of debate concerning whether NII deposition plays a causative role in the pathogenesis of cell death in HD. Interestingly, a recent study reports that in HD cortex and striatum inclusion deposition does not mirror the pattern of cell death in the disease (59). NII and CI are seen in NADPH-d neurons spared in the disease, but are not found in acetylcholinesterase- and choline acetyltransferase-positive interneurons, suggest-

ing that NII formation is not critical for cell death mechanisms. Furthermore, although expression of human mutant huntingtin in transgenic mouse models of HD is associated with the development of movement disorders and deposition of huntingtin aggregates reminiscent of HD, no murine model reported to date exhibits the selective neuronal death which characterizes HD (30, 66, 102). There is *in vitro* evidence that expanded CAG repeats can induce huntingtin aggregation and cell death in transfected cerebellar granule cell cultures (72). In addition, transfection of a human mutant huntingtin fragment into *Drosophila* eye cells induced CAG repeat-dependent photoreceptor degeneration and death, putatively via apoptotic mechanisms (54). However, Saudou and colleagues (85) recently reported cell-selective neurodegeneration resembling apoptotic cell death in cultured striatal (but not hippocampal) neurons transfected with a human mutant huntingtin fragment, independent of the presence of intranuclear inclusions, suggesting that NII deposition may reflect a protective mechanism within cells. This proposition is supported by observations that suppression of NII deposition led to increased cell death in this neuronal population.

Although neither full length huntingtin or huntingtin fragments have yet been found in mitochondria, an effect on mitochondrial function cannot be ruled out. For instance, huntingtin may play a role in mitochondrial trafficking, or alternatively NII may influence nuclear transcription and thus affect the expression of nuclear-encoded proteins including subunits of mitochondrial complex II. The latter is of particular note since complex II activity is impaired in affected brain areas in HD.

Conclusions

Multiple lines of evidence indicate that oxidative stress and energetic defects may play roles in the etiology of selective neuronal death in HD. To date it has been impossible to determine whether bioenergetic dysfunction or oxidative damage are causative factors in the disease process, or merely occur secondarily to neuronal loss in HD. Furthermore, although the genetic mutation responsible for the phenotype in HD is known and its protein product identified, the mechanism whereby abnormal huntingtin protein leads to region-specific cell death is unknown. The recent development of transgenic mouse models of HD has provided a valuable opportunity to elucidate the pathologic sequence of events culminating in disease phenotype and cell death associated with the HD gene mutation, which is impossible to determine from end-stage post-mortem tissue.

Several transgenic mouse models of HD have recently been developed. Of these the best characterized to date are R6/2 mice, developed by inserting a transgene containing an N-terminal fragment of human mutant huntingtin carrying CAG repeat expansions of 145-150 units (66). Mice exhibit a rapidly progressing neurological phenotype, with onset at approximately 2 months of age and lifespan of up to 17 weeks. They develop a movement disorder involving resting tremor, shuddering, stereotypic grooming behaviour, and epileptic seizures, and lose body weight from 8 weeks of age. Brain weight is reduced approximately 20% at 12 weeks, relative to normal littermate controls. Neuronal intranuclear inclusions (NII) of the transgene protein are evident by 3-4 weeks of age (30). It is anticipated that the investigation of oxidative stress markers in this and other transgenic mouse models of HD will soon provide vital insight into the etiologic role of oxidative damage in mechanisms of neuronal death in HD.

Acknowledgements

This work was supported in part by NIH grant NS35255 (RJF), by the Huntington's Disease Society of America (SEB), and by NS16367 (Huntington's Disease Center Without Walls, MFB).

References

1. Albin RL, Reiner A, Anderson KD, Penney JB, Young AB (1990) Striatal and nigral neuron subpopulations in rigid Huntington's disease: Implications for the functional anatomy of chorea and rigidity-akinesia. *Ann Neurol* 27: 357-365
2. Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12: 366-375
3. Albin RL, Greenamyre JT (1992) Alternative excitotoxic hypotheses. *Neurology* 42: 733-738
4. Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* 13: 266-271
5. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B, Kalchman MA et al. (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat Genet* 4: 398-403
6. Beal MF. (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illness? *Ann Neurol* 31: 119-130
7. Beal MF (1994) Neurochemistry and toxin models in Huntington's disease. *Curr Op Neurol* 7: 542-547
8. Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* 321: 168-171

9. Beal MF, Ellison DW, Mazurek MF, Swartz KJ, Malloy JR, Bird ED, Martin JB (1988) A detailed examination of substance P in pathologically graded cases of Huntington's disease. *J Neurol Sci* 84: 51-61
10. Beal MF, Swartz KJ, Hyman BT, Storey E. Finn SF and Koroshetz W. (1991b) Aminooxyacetic acid results in excitotoxin lesions by a novel indirect mechanism. *J Neurochem* 57: 1068-1073
11. Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, Storey E, Srivastava R, Rosen BR, Hymen BT (1993a) Neurochemical and histologic characterization of excitotoxic lesions produced by mitochondrial toxin 3-nitropropionic acid. *J Neurosci* 13: 4181-4192
12. Beal MF, Hyman BT and Koroshetz W. (1993b) Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? *Trends Neurosci* 16: 125-131
13. Beal MF, Henshaw DR, Jenkins BG, Rosen BR, Schulz JB. (1994) Coenzyme Q10 and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate. *Ann Neurol* 36: 882-888
14. Beal MF, Ferrante RJ, Henshaw R, Matthews RT, Chan PH, Kowall NW, Epstein CJ, Schulz JB (1995) 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. *J Neurochem* 65: 919-922
15. Blaustein MP (1988) Calcium transport and buffering in neurons. *Trends Neurosci* 11: 438-443
16. Bolanos JP, Heales SJR, Land JM, Clark, JB (1995) Effect of peroxynitrite on the mitochondrial respiratory chain: Differential susceptibility of neurones and astrocytes in primary culture. *J Neurochem* 64: 1965-1972
17. Braak H, Braak E (1992) Allocortical involvement in Huntington's disease. *Neuropathol App Neurobiol* 18: 539-547
18. Brennan WA, Bird ED, Aprille JR. (1985) Regional mitochondrial respiratory activity in Huntington's disease brain. *J Neurochem* 44: 1948-1950
19. Bresolin N, Bet L, Binda A, Moggio M, Comi G, Nador F, Ferrante C, Carenzi A, Scarlato G (1988) Clinical and biochemical correlations in mitochondrial myopathies treated with coenzyme Q10. *Neurology* 38: 892-899
20. Brouillet EP, Jekins BG, Hyman BT, Ferrante, RJ, Kowall NW, Srivastava R, Samanta-Roy D, Rosen BR, Beal MF (1993) Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *J Neurochem* 60: 356-359
21. Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc Natl Acad Sci USA* 92: 7105-7109
22. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MMK, Bird ED, Beal MF (1997) Oxidative damage and metabolic dysfunction in Huntington's Disease: Selective vulnerability of the basal ganglia. *Annals Neurol* 41: 646-653
23. Browne SE, Beal MF (1994) Oxidative damage and mitochondrial dysfunction in neuro-degenerative diseases. *Biochem Soc Trans* 22: 1002-1006
24. Burke JR, Enghild JJ, Martin ME, Jou Y-S, Myers RM, Roses AD, Vance VM, Strittmatter WJ (1996) Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nature Med* 2: 347-350
25. Butterworth J, Yates CM, Reynolds GP (1985) Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase, and α-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. *J Neurol Sci* 67: 161-171
26. Butterworth NJ, Williams L, Bullock JY, Love DR, Faull RLM, Dragunow M. (1998) Trinucleotide (CAG) repeat length is positively correlated with the degree of DNA fragmentation in Huntington's disease striatum. *Neurosci* 87: 49-53
27. Calabresi P, Centonze D, Pisani A, Sancesario G, Gubellini P, Marfia GA, Bernardi G (1998) Striatal spiny neurons and cholinergic interneurons express differential ionotropic glutamatergic responses and vulnerability: implications for ischemia and Huntington's disease. *Ann Neurol* 43: 586-597
28. Choi DW (1988) Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci* 11: 465-469
29. Cooper AJL, Sheu K-FR, Burke JR, Onodera O, Strittmatter WJ, Roses AD, Blass JP. (1997) Transglutaminase-catalyzed inactivation of glyceraldehyde 3-phosphate dehydrogenase and a-ketoglutarate dehydrogenase complex by polyglutamine domains of pathological length. *Proc Natl Acad Sci USA*. 94: 12604-12609
30. Davies SW, Turmaine M, Cozens B, DiFiglia M, Sharp A, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates G. (1997) Formation of neuronal intranuclear inclusions (NII) underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90: 537-548.
31. de la Monte SM, Vonsattel JP, Richardson Jr EP (1988) Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. *J Neuropath Exp Neurol* 47: 516-525
32. DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, Martin E, Vonsattel J-P, Carraway R, Reeves SA, Boyce FM, Aronin N (1995) Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* 14: 1075-1081
33. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel J-P, Aronin N. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 277: 1990-1993
34. Driggers WJ, Holmquist GP, LeDoux SP, Wilson GL (1997) Mapping frequencies of endogenous oxidative damage and the kinetic response to oxidative stress in a region of rat mtDNA. *Nucleic Acids Res* 25: 4362-9

35. Dugan LL, Sensi SL, Canzoniero LM, Handran SD, Rothman SM, Lin TS, Goldberg MP, Choi DW (1995) Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. *J Neurosci* 15: 6377-6388

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

37. Duyao MP, Auerbach AB, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel J-P, Gusella JF, Joyner AL, MacDonald ME (1995) Inactivation of the mouse Huntington's disease gene homolog (*Hdh*). *Science* 269: 407-410

38. Dykens JA (1994) Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^{+}: implications for neurodegeneration. *J Neurochem* 63: 584-91

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

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

41. Ferrante RJ, Kowall NW, Hersch SM, Brown rh, Beal MF (1996) Immunohistochemical localization of markers of oxidative injury in Huntington's disease. *Soc Neurosci* 22: 92.5

42. Fraga CG, Shigenaga MK, Park J-W, Degan P, Ames BN (1990) Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci USA* 87: 4533-4537

43. Furtado S, Suchowersky O, Rewcastle B, Graham L, Klimek ML, Garber A. (1996) Relationship between trinucleotide repeats and neuropathological changes in Huntington's disease. *Ann Neurol* 39: 132-136

44. Goebel HH, Heipertz R, Scholz W, Iqbal K, Tellez-Nagel I. (1978) Juvenile Huntington chorea: clinical, ultrastructural, and biochemical studies. *Neurology* 28: 23-31

45. Gonzalez-Zulueta M, Ensz LM, Mukhina G, Lebovitz RM, Zwacka RM, Engelhardt JF, Oberley LW, Dawson VL, Dawson TM. (1998) Manganese superoxide dismutase protects nNOS neurons from NMDA and nitric oxide-mediated neurotoxicity. *J Neurosci* 18: 2040-2055

46. Graveland GA, Williams RS, DiFiglia M (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science* 227: 770-773 n

47. Graybiel AM, Aosaki T, Flaherty AW, Kimura M (1994) The basal ganglia and adaptive motor control. *Science* 265: 1826-1831

48. Greene JG, Porter RH, Eller RV, Greenamyre JT (1993) Inhibition of succinate dehydrogenase by malonic acid produces an "excitotoxic" lesion in rat striatum. *J Neurochem* 61: 1151-1154

49. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AHV (1996) Mitochondrial defect in Huntington's disease caudate nucleus. *Ann Neurol* 39: 385-389

50. Gutekunst C-A, Levey AI, Heilman CJ, Whaley WL, Yi H, Nash NR, Rees HD, Madden JJ, Hersch SM (1995) Identification and localization of huntingtin in brain and human lympho-blastoid cell lines with anti-fusion protein antibodies. *Proc Natl Acad Sci USA* 92: 8710-8714

51. Hemmer W, Wallimann T. (1993) Functional aspects of creatine kinase in brain. *Dev Neurosci* 15: 249-260

52. Ihara Y, Namba R, Kuroda S, Sato T, Shirabe T (1989) Mitochondrial encephalomyopathy (MELAS): pathological study and successful therapy withcoenzyme Q10 and idebenone. *J Neurol Sci* 90: 263-271

53. Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, Beckman JS (1992) Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophys* 298: 431-7

54. Jackson GR, Salecker I, Schulze KL, Dong X, Yao X, Arnheim N, Faber P, MacDonald ME, Zipursky SL (1998) Age- and polyglutamine length-dependent degeneration of drosophila photoreceptor neurons induced by human huntingtin transgenes. *Soc Neurosci* 24: 205.2

55. Jenkins BG, Koroshetz W, Beal MF, Rosen B (1993) Evidence for an energy metabolism defect in Huntington's disease using localized proton spectroscopy. *Neurology* 43: 2689-2695

56. Jenkins BG, Rosas HD, Chen Y-CI, Makabe T, Myers R, MacDonald M, Rosen BR, Beal MF, Koroshetz WJ (1998) 1H-NMR spectroscopy studies of Huntington's disease. *Neurology* 50: 1357-1365

57. Koh J-Y, Peters S, Choi DW (1986) Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. *Science* 234: 73-76

58. Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF (1997) Energy metabolism defects in Huntington's disease and possible therapy with coenzyme Q_{10}. *Ann Neurol* 41: 160-165

59. Kuemmerle S, Klein AM, Gutekunst C-A, Li X-J, Li S-H, Hersch SM, Ferrante R (1998) Distribution of huntingtin aggregation in spared and vulnerable neurons in Huntington's disease. *Soc Neurosci* 24: 380.14

60. Kuhl DE, Markham CH, Metter EJ, Riege WH, Phelps ME, Mazziotta JC (1985) Local cerebral glucose utilization in symptomatic and presymptomatic Huntington's disease. *Research Publications - Association for Research in Nervous and Mental Disease* 63: 199-209

61. Kuwert T, Lange HW, Langer K-J, Herzog H, Aulich A, Feinendegen LE (1990) Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. *Brain* 113: 1405-1423

62. Landwehrmeyer GB, McNeil SM, Dure LS, Ge P, Aizawa H, Huang Q, Ambrose CM, Duyao MP, Bird ED, Bonilla E, de Young M, Avila-Gonzales AJ, Wexler NS, DiFiglia M, Gusella JF, MacDonald ME, Penney JB, Young AB and Vonsattel J-P (1995) Huntington's disease gene: Regional and cellular expression in brain of normal and affected individuals. *Ann Neurol* 37: 218-230

63. Li X-J, Li S-H, Sharp AH, Nucifora FC Jr., Schilling G, Lanahan A, Worley P, Snyder SH, Ross CA (1995) Huntingtin-associated protein enriched in brain with implicatins for pathology. *Nature* 378: 398-402

64. Linnane AW, Marzuki S, Ozawa T, Tanaka M (1989) Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* 1(8639): 642-645

65. Ludolph AC, He F, Spencer PS, Hammerstad J, Sabri M. (1990) 3-nitropropionic acid: exogenous animal neurotoxin and possible human striatal toxin. *Can. J. Neurol. Sci.* 18: 492-498

66. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates G (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 87: 493-506

67. Marnett LJ, Buck J, Tuttle MA, Basu AK, Bull AW (1985) Distribution and oxidation of malondialdehyde in mice. *Prostaglandins* 30: 241-254

68. Matsumara M, Tremblay L, Richard H, Filion M (1995) Activity of pallidal neurons in the monkey during dyskinesia induced by injection of bicuculline in the external pallidum. *Neurosci* 65: 59-70

69. Matthews RT, Yang L, Jenkins BJ, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, Beal MF (1998) Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* 18: 156-163

70. Mazziotta JC, Phelps ME, Pahl JJ, Huang SC, Baxter LR, Riege WH, Hoffman JM, Kuhl DE, Lanto AB, Wapenski JA, Markham CH (1987) Reduced cerebral glucose metabolism in asymptomatic patients at risk for Huntington's disease. *New Eng J Med* 316: 357-362

71. May PC, Gray PN (1985) The mechanism of glutamate-induced degeneration of cultured Huntington's disease and control fibroblasts. *J Neurol Sci* 70: 101-112

72. Moulder KL, Onodera O, Burke J, Strittmatter WJ, Johnson Jr EM (1998) Polyglutamine containing proteins produce length-dependent aggregation and death in cultured cerebellar granule cells. *Soc Neurosci* 24: 379.5

73. Myers RH, Vonsattel JP, Paskevich PA, Kiely DK, Stevens TJ, Cupples LA, Richardson EP Jr, Bird ED (1991) Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J Neuropathol Exp Neurol.* 50: 729-742

74. Nakano M, Gotoh S (1992) Accumulation of cardiac lipofuscin depends on metabolic rate of mammals. *J Gerontol* 47: B126-129

75. Nicoletti F, Bruno V, Copani A, Casabona G, Knopfel T (1996) Metabotropic glutamate receptors: A new target for the therapy of neurodegenerative disorders? *Trends Neurosci* 19: 267-271

76. Novelli A, Reilly JA, Lysko PG, Henneberry RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res* 451: 205-212

77. O'Brien CF, Miller C, Goldblatt D, Welle S, Forbes G, Lipinski B, Panzik J, Peck R, Plumb S, Oakes D, Kurlan R, Shoulson I (1990) Extraneural metabolism in early Huntington's disease. *Ann Neurol* 28: 300-301

78. Paulson HL, Perez MK, Trottier Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN (1997) Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* 19: 333-344

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

80. Portera-Cailliau C, Hedreen JC, Price DL, Koliatsos VE (1995) Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. J Neurosci 15: 3775-3787

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

82. Richfield EK, Maguire-Zeiss KA, Cox C, Gilmore J, Voorn P (1995) Reduced expression of preproenkephalin in striatal neurons from Huntington's disease patients. *Ann Neurol* 37: 335-343

83. Ross CA (1995) When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* 15:493-6

84. Sapp E, Ge P, Aizawa H, Bird E, Penney J, Young AB, Vonsattel JP, DiFiglia M (1995) Evidence for a preferential loss of enkephalin immunoreactivity in the external globus pallidus in low grade Huntington's disease using high resolution image analysis. *Neurosci* 64: 397-404

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

86. Scherzinger E, Kurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, BatesGP, Davies SW, Lehrach H, Wanker EE (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like aggregates in vitro and in vivo. *Cell* 90: 549-558

87. Schulz JB, Henshaw DR, Jenkins BG, Ferrante RJ, Kowall NW, Rosen BR and Beal MF (1994) 3-Acetylpyridine produces age-dependent excitotoxic lesions in rat striatum. *J Cereb Blood Flow Metab* 14: 1024-1029

88. Schulz JB, Henshaw DR, MacGarvey U and Beal MF. (1996a) Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. *Neurochem Intl* 29: 167-171

89. Schulz JB, Matthews RT, Henshaw DR, Beal MF (1996b) Neuroprotective strategies for treatment of lesions produced by mitochondrial toxins: implications for neurodegenerative diseases. *Neurosci* 71: 1043-1048

90. Sharp NH, Loev SJ, Schilling G, Li S-H, Li X-J, Bao J, Wagster MV, Kotzuk JA, Steiner JP, Lo A, Hedreen J, Sisodia S, Snyder SH, Dawson TM, Ryugo DK, Ross CA (1995) Widespread expression of the Huntington's disease gene (IT-15) protein product. *Neuron* 14: 1065-1074

91. Sohal RS, Marzabadi MR, Galaris D, Brunk UT (1989) Effect of ambient oxygen concentration on lipofuscin accumulation in cultured rat heart myocytes—a novel in vitro model of lipofuscinogenesis. *Free Radic Biol Med* 6: 23-30
92. Sotrel A, Paskevich PA, Kiely DK, Bird ED, Willimas RS, Myers RH (1991) Morphometric analysis of the prefrontal cortex in Huntington's disease. *Neurology* 41: 1117-1123
93. Stadtman ER (1992) Protein oxidation and aging. *Science* 257: 1220-1224
94. Tellez-Nagel I, Johnson AB, Terry RD (1995) Studies on brain biopsies of patients with Huntington's chorea. *J Neuropathol Exp Neurol* 33: 308-332
95. Terman A, Brunk UT (1998) Lipofuscin: mechanisms of formation and increase with age. *APMIS* 106: 265-276
96. Trottier Y, Devys D, Imbert G, Saudou F, An I, Lutz Y, Weber C, Agid Y, Hirsch EC, Mandel JL (1995) Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nat Genet* 10: 104-110
97. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosome. *Cell* 72: 971-983
98. Uemera Y, Kowall NW, Beal MF (1990) Relative sparing of NADPH-diaphorase-somatostatin-neuropeptide Y neurons in ischemic gerbil striatum. *Ann Neurol* 27: 620-625
99. Vonsattel J-P, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP (1985) Neuropathological classification of Huntington's disease. *J Neuropath Exp Neurol* 44: 559-577
100. Vonsattel JPG, DiFiglia M (1998) Huntington Disease. *J Neuropathol Exp Neurol* 57: 369-384
101. Weeks RA, Harding AE, Brooks DJ (1996) Striatal D1 and D2 dopamine receptor loss in asymptomatic mutation carriers of Huntington's disease. *Ann Neurol* 40: 49-54
102. White JK, Auerbach W, Duyao MP, Vonsattel J-P, Gusella JF, Joyner AL, MacDonald ME (1997) Huntingtin function is required for mouse brain development and is not impaired by the Huntington's disease CAG expansion mutation. *Nat Genet* 17: 404-410
103. Young AB, Greenamyre JT, Hollingsworth Z, Albin R, D'Amato C, Shoulson I, Penney JB (1988) NMDA receptor losses in putamen from patients with Huntington's disease. *Science* 241: 981-3
104. Zeevalk GD, Nicklas WJ (1991) Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. *J Pharm Exp Ther* 257: 870-878
105. Zhang J, Dawson VL, Dawson TM, Snyder SH (1994) Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* 263: 687-689