

Hereditary Disease Foundation

***Planning for the Millennium: Setting the Research
Agenda for the Countdown Year***

The Darina Wiesel Memorial Workshop

January 9 and 10, 1999
Santa Monica, California

Prepared by Erik Schweitzer

HEREDITARY DISEASE FOUNDATION WORKSHOP

“Planning for the Millennium:
Setting the Research Agenda for the Countdown Year”

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Planning for the Millennium: Setting the Research Agenda for the Countdown Year The Darina Wiesel Memorial Workshop

report prepared by Erik S. Schweitzer, M.D., Ph.D.

On January 9 and 10, 1999, the Hereditary Disease Foundation held a workshop to bring together investigators pursuing an understanding of and cure for Huntington's Disease (HD). Participants presented recent results of their investigations, and discussed the significance of these findings, the important questions outstanding, and productive approaches to addressing the significant issues that remain. There were also moving testaments to the human face of HD, including a tribute and written testimonial by Mark Wiesel, the husband of the late Darina Wiesel, who died from complications of HD in March 1998; and a discussion with another family affected by HD. In addition, participants attended a dinner at the Geffen Contemporary gallery, celebrating the 30th anniversary of the Hereditary Disease Foundation, the 90th birthday of founder Dr. Milton Wexler, and the imminent arrival of the new millennium.

Significance

Huntington's disease is an example of a class of neurological diseases caused by expansions of a triplicate nucleotide repeat coding for glutamine residues within the coding sequence for specific proteins. The identity of the specific protein that contains this expanded polyglutamine region determines the particular manifestations and cellular targets of the disease. Huntington's disease is caused by an expansion of the CAG repeat in exon 1 of huntingtin, a 350 kD protein expressed widely throughout the nervous system and body, but whose function is presently unknown. Patients typically develop disturbances in cognitive and emotional function, uncontrollable movements, and mental decline without remission, progressing to death over a 10-20 year course.

These changes in brain function are accompanied by marked degeneration of medium-sized spiny neurons in the striatum, as well as cell loss in the cerebral. In addition to neuronal cell loss and shrinkage of the brain, the pathological appearance of the brain in HD is characterized by the presence, within some surviving neurons in the striatum and cerebral cortex, of intranuclear inclusions.

HD exhibits an autosomal dominant inheritance, and until recently most patients had no way to know whether they carried the disease-causing mutation until after childbearing age. The symptoms of HD vary in age of onset and severity; earlier and more rapidly progressing symptoms are correlated with larger expansions of the CAG repeat. There is a tendency for the length of the CAG repeat to increase in successive generations, leading to a progressively earlier onset of the disease. With the identification of the genetic defect responsible for HD, the generation of transgenic mice expressing the mutant huntingtin gene, and the establishment of cell culture systems permitting the examining of the cell biology of huntingtin, we have entered a new era in understanding and ultimately eradicating HD.

Scientific Discussion

The presentation and discussion of experimental results focussed on several themes. Within each theme, the participants actively discussed the most recent findings in that area, the deficiencies that remain to be addressed, and the most important directions to pursue in the near future. These major areas included the following:

- 1) transgenic models for HD (mostly mouse, but now also including *Drosophila*);
- 2) characteristics of neuronal dysfunction and death in HD;
- 3) the nature of aggregates and their role in the disease process;
- 4) the role of protein degradation and turnover in HD and other polyglutamine diseases;
- 5) cell culture and peripheral cells as models of HD; and
- 6) markers, other than cell death, for the progression and symptomatology of HD.

1) Transgenic models for HD

Considerable information has now accumulated about a variety of transgenic mouse strains for HD. These include mice expressing various sizes of expanded polyglutamine tracts, inserted into either full-length huntingtin or exon 1. Transgenic mice are now also available as models for spinal and bulbar muscular atrophy (polyglutamine expansion in the androgen receptor), dentatorubral-pallidoluysian atrophy (DRPLA) (polyglutamine expansion in atrophin-1), and SCA-1 (polyglutamine expansion in ataxin-1). These various mouse strains, and some of their most important characteristics, are shown in Table 1.

Transgenic mice have been valuable models for examining the pathogenesis of HD. They exhibit weight loss, ataxia, involuntary movements, seizures, and early death. As reported by Stephen Davies, there is considerable cell loss in the dorsal striatum, anterior cingulate cortex, and Purkinje cells of the cerebellum at the late stages, as evidenced by fewer cells and the presence of shrunken cells. However, this cell loss is not readily apparent until the tissue is examined in the electron microscope, where cells have a peculiar but characteristic appearance. The cells become shrunken and dark when stained with OsO_4 , a characteristic he termed "dark cell degeneration." These cells appear after 14-17 weeks, and do not fit the classical description of apoptotic cells. They appear to be dying over a prolonged period (~3 weeks), never exhibit TUNEL positivity, and never form apoptotic bodies.

Because huntingtin is expressed throughout the body, there remains a puzzle as to why, or whether, pathology is only expressed in neurons. One logical place to look for similar pathological changes is the endocrine pancreas, since pancreatic cells share many characteristics with neurons. Indeed, some transgenic HD mice show an increased tendency for the development of diabetes. This is a late manifestation of the disease, and is seen only in association with truncated, not full-length, huntingtin. Stephen Dunnett expressed the belief that the weight loss observed in HD mice is not secondary to diabetes, but is due to the appearance of multiple motor deficits after 5-7 weeks of age, resulting in a difficulty in rearing up to obtain food from conventional mouse cages. All of Steve Dunnett's behavioral experiments were done collaboratively with Jenny Morton's laboratory.

One possible etiology for diabetes in HD mice is that neuronal death leads to the release of GAD (glutamic acid decarboxylase), which causes an autoimmune

reaction, which in turn attacks the pancreas. This seems especially plausible since pancreatic β cells, along with neurons, express GAD, and since antibodies against GAD are early markers for diabetes in humans, and may be causal. However, the absence of an inflammatory response in the pancreas of HD mice argues against such an explanation. In addition, meeting participants differed in their opinions about whether human HD is associated with diabetes, and if so what kind. It was suggested that the type of diabetes associated with human HD is type II, which does not involve a decreased ability of the β cells to release insulin, but rather a decreased sensitivity of insulin receptors in peripheral tissues. Nancy Wexler stated that the Huntington's Disease Study Group did not identify any association of diabetes with human HD; such an association might be revealed by more sensitive analyses.

There were several other observations offered from transgenic HD mice. One characteristic of human HD that is shared by the transgenic mice is the tendency for an increase in the CAG repeat length with successive generations. Anne Young mentioned that there is a decrease in the mRNA for many neurotransmitter receptors including D1, D2, and adenosine receptors. There is, however, no change in the mRNA levels for the two isoforms of GAD (GAD₆₅ and GAD₆₇). Marie-Francoise Chesselet pointed out that enkephalin mRNA levels drop 80%, but that there is no corresponding decrease in the level of enkephalin peptides. In contrast, mRNA levels for substance P are not decreased. The basis for this dissociation between mRNA and peptide levels remains unexplained. There are also metabolic deficits in the HD mice, including a ~50% drop in N-acetyl aspartate (NAA) levels, which may be an early marker for neuronal damage.

Nancy Bonini described an exciting new approach using mutants in *Drosophila* to investigate the mechanisms of pathogenesis in polyglutamine diseases, particularly by looking for revertants that protect the flies from the toxic effects of expanded huntingtin. She is proceeding along a number of different lines to develop *Drosophila* models for human neurodegenerative disease, and to define genes that can delay or prevent neurodegeneration. She has already developed a fly model for polyglutamine-repeat disease using a form of the Machado-Joseph disease protein. She is now developing a model using forms of the Huntington's disease protein, as well as other polyglutamine-repeat disease proteins. She is addressing issues of tissue specificity, and comparison of the toxicity and tissue specificity of truncated forms of the various proteins with full-length forms.

She is using these *Drosophila* models in order to define genes that can delay or prevent polyglutamine-induced neural degeneration. Toward this end, she is testing specific genes suggested by studies in vertebrates, including chaperone proteins and proteins of the ubiquitin proteolytic pathways. In addition, she is performing genetic screens in order to define genes that can ameliorate degeneration induced by

polyglutamine-protein in *Drosophila*. The goal of her research is to define these genes in flies, and then test the defined proteins for their ability to ameliorate the effects of the toxic proteins in vertebrates.

For the future, mouse models will continue to provide valuable information about the pathogenesis and symptomatology of HD. Transgenic mice mimic the human disease in a remarkable number of aspects. They will also provide important tools

for testing therapeutic agents, independent of continuing advances in our understanding of the mechanisms that underlie the disease process.

2) Characteristics of neuronal dysfunction and death in HD.

Both HD mice and cell culture models have provided new information about the characteristics of the neuronal cell death that results from the expression of mutant huntingtin. The nature of this cell death was the subject of much animated discussion. Although there was an earlier impression that cells were dying by apoptosis, a consensus appeared that the cell death observed both in animals and in culture does not fit the rigorous criteria established for true apoptosis. Whether it is legitimate to refer to neuronal death in HD as "atypical apoptosis" or "apoptosis-like", or whether it is completely different depends in part on one's view about the narrowness of the term apoptosis. Most strictly, apoptosis is characterized by rapid cell death accompanied by fragmentation of the nucleus, activation of caspases, DNA fragmentation (TUNEL positivity), and the formation of dense apoptotic bodies. However, there is a growing appreciation in a variety of fields that there is a whole spectrum of modes of cell death between apoptosis and necrosis, and it may be too simplistic to expect a given system to match all the classical criteria. A more pragmatic approach is to ask what aspects of the cell death in HD are informative in terms of understanding the underlying disease mechanisms.

As characterized by Stephen Davies, the dying cells in HD mice appear shrunken and dark when stained with OsO_4 . The cells appear to die over a prolonged time, and are never TUNEL positive, nor form apoptotic bodies. The mitochondria and Golgi apparatuses transiently open up, and then re-close. There is no evidence of reactive astrocytes, and instead the end stage of neuronal cell death is the cell's becoming engulfed by atypical astrocytes (GFAP⁺). He characterized the cell death as being neither apoptotic nor necrotic. A similar pathological appearance is seen in postmortem human HD brains.

Charles Wilson suggested that the extent to which neuronal cell death is important or necessary in human HD remains uncertain. While historically there has been a tendency for the HDF to view this as an important disease parameter, this may be misleading. Although shrinkage of the striatum can be observed in human patients by MRI, there are major irregularities in the extent of cell death. While juvenile-onset HD is accompanied by cerebellar Purkinje cell death, it is not clear that this cell death is a direct result of the disease process. Cell loss is especially prominent along the midline of the vermis, in an area that is not typically affected by neuroleptics or epilepsy, although this type of cell loss can be caused by ethanol.

Leslie Thompson reported a remarkably similar type of cell morphology in cultured PC12 cells expressing mutant huntingtin. In these cells, expression of expanded glutamine huntingtin (truncated exon 1 under ecdysone control) leads to successive waves of aggregate formation which seems, in many cases, to be followed by cell senescence or death. These cells look dark, and may contain aggregates. Naked aggregates also appear in the dish; it remains to be determined whether these are extruded from living cells in an attempt to rid the cells of aggregated huntingtin, or are the remnants of cells that have died and been broken down, leaving only the indigestible aggregates.

Important directions for the future include determining whether this type of cell death can reasonably be considered some variant of apoptosis, or is some

completely different form of cell death. Rather than trying to fit this phenomenon into existing categories, it may be more useful to analyze the steps and mechanisms responsible for the cell death associated with HD, in the hope of understanding its significance, cause, and/or finding an intervention that might prevent it.

3) The nature of aggregates and their role in the disease process.

The importance of intracellular aggregates has been, and remains a source of debate. One extreme position is that the aggregates are necessary and sufficient to cause the pathological changes that result in cell dysfunction and eventual death. At the opposite extreme is the possibility, articulated by Alfred Goldberg, that the aggregates are actually protective; that by removing mutant huntingtin from the cell cytoplasm, they decrease the toxic effects of the soluble protein. An alternative to either of these extremes is the possibility that the aggregates play no role whatsoever, and are merely epiphenomena that accompany but do not contribute to the pathogenesis of HD.

Michael Greenberg and Christopher Ross discussed the findings that expression of a dominant negative ubiquitinating enzyme prevents the formation of nuclear aggregates, but does not prevent the cell death caused by mutant huntingtin, suggesting that visible aggregates are not essential for toxicity. However, there was some discussion of the crude definition of aggregates, since significant "micro-inclusions," or even multimers of huntingtin could form without the appearance of aggregates observable in the light microscope. Nevertheless, HD mice also show a dissociation between aggregates and cell death: hippocampal neurons have just as many inclusions as the striatum, but there is not cell death in the former location. There was a general consensus that there has not yet been an unambiguous answer to the question of whether aggregates are necessary or not for cellular pathology, and further experiments should be pursued to pin down this important question.

There was some discussion of the desirability of demonstrating a direct toxic effect of huntingtin aggregates in a way analogous to that done with Alzheimers disease, where exogenous application of amyloid is toxic to cells. However, such an approach is probably impractical for huntingtin aggregates, because they act intracellularly, and there are severe technical obstacles to microinjecting aggregates into single cells and monitoring cell toxicity. Nevertheless, there was considerable sentiment that purification of aggregates, perhaps from Thompson's cell culture system, is a desirable goal, even if only to define more fully their composition. Marie-Francoise Chesselet noted that extracellular blobs have been observed by M. Levine at UCLA using infrared microscopy to examine live slices of striatum from HD (Bates) mice by. These formations are not observed in fixed tissue, and it is not clear whether these blobs are huntingtin aggregates.

David Housman injected a note of caution into attempts to purify aggregates, by pointing out that the intracellular aggregates of huntingtin observed within PC12 cells are remarkably resistant to disruption. His observations on huntingtin tagged with GFP indicate that treatment with SDS does not disrupt the aggregates; moreover, it does not even denature the GFP contained within the aggregates, as indicated by the continued bright fluorescence of the aggregates after such treatment. Although not quantitative, such observations suggest that it will be difficult to analyze the contents of the aggregates by conventional biochemical means. A particularly

strong denaturation agent, HFIP, may be useful in attempting to break up such aggregates. Alfred Goldberg made another cautionary observation: the aggregates that can be purified from cell culture or other sources may not be identical with those found in striatal cells. He also pointed out that interfering with protein degradation, by adding inhibitors of proteasomes, will itself cause the appearance of protein aggregates.

In contrast to the uncertainty about the role of aggregates in cellular pathology, nuclear localization of mutant huntingtin seems to be essential for its toxicity. Thus in N2A neuroblastoma cells, the addition of a nuclear export signal to mutant huntingtin decreases its toxicity, while fusion with a nuclear import signal increases toxicity.

Questions about the role of aggregates in HD brought up the more fundamental question of whether cell death is either an essential part of the disease, or a reasonable marker for disease progression either in the brain or in model systems. There is a growing sense that we need other markers besides intranuclear aggregates and cell death to understand and follow the disease.

4) The role of protein degradation and turnover in HD.

One appealing model that connects the necessity for nuclear localization of huntingtin with the general toxicity of polyglutamine tracts is the possibility that extended stretches of polyglutamine poison the proteasomes, and interfere with the normal turnover of proteins, particularly nuclear regulatory and transcriptional factors. This turnover is normally carried out by proteasomes within the cytoplasm and nucleus, and there is accumulating evidence that the proteasome system is involved in some way with the pathogenesis of HD. Both ubiquitin and proteasome components are found associated with the aggregates of huntingtin, suggesting some sort of abortive attempt by the cell to degrade the mutant huntingtin.

Alfred Goldberg described how bacterial proteasomes degrade proteins in a processive fashion, and if an indigestible region of the protein is encountered, the protein falls off, binds to chaperone proteins, and unfolds. It is not clear whether mammalian proteasomes behave in the same fashion, or whether an indigestible region of a protein could become permanently stuck in the interior of the proteasome, leading to a structurally intact but functionally inactive proteasome.

There was discussion of the observation that expression of mutant huntingtin leads to the appearance of a new fragment of ~100kD, which does not appear with wild type huntingtin. Although this has been taken as evidence for the appearance of a new proteolytic activity, there are several alternative explanations for such a fragment. It may result from proteolysis that occurs after cell death or disruption, for example by endopeptidase Y. Alternatively, this fragment could be the result of a shorter transcription or translation product, such as one produced by premature termination of the huntingtin mRNA. Thirdly, such a fragment could result from the loss of normal proteolytic activity rather than the appearance of new proteolytic activity, and represent an abnormally stable form of a fragment that occurs transiently as a normal intermediate in the degradation of wild type huntingtin. There was a general sense that it is important to establish the nature of this 100 kD fragment, and that pulse-chase experiments should be carried out to determine its origin and fate.

Alfred Goldberg also described experiments in which CDC34, a dominant negative mutation of a specific ubiquitination enzyme prevented aggregate formation but did not cause any change in the toxicity of mutant huntingtin. This enzyme is probably fairly specific for cell cycle proteins, and should not affect overall ubiquitination and general protein turnover in the cell.

Jon Wood and Gabriele Schilling in Chris Ross's lab, in collaboration with Dave Borchelt's lab, have generated a transgenic mouse model of DRPLA, a poly-Q disease very similar to HD. The mice (see Table 1 for phenotype) have accumulation of what appears to be a proteolytically processed portion of atrophin (the DRPLA gene product). This fragment consists of about half of the molecule, including the N-terminus, and the poly-Q tract, and is highly enriched in the nucleus. It does not correspond to any known caspase fragment of atrophin, and is present as early as one month postnatally, so it is not likely to be a consequence of caspase cleavage secondary to cell death.

Gabriele Schilling in Chris Ross's lab, in collaboration with Dave Borchelt's lab, is continuing to observe both intranuclear inclusions and diffuse nuclear labeling for huntingtin in their transgenic model of HD using a short N-terminal fragment of huntingtin (see Table 1 for phenotype). In collaboration with Steve Hersch's lab, they now have preliminary evidence for neuronal degeneration in the striatum of these mice. Degenerating cells do not necessarily have huntingtin inclusions, though they may have microaggregates.

Matt Peters and Fred Nucifora in Chris Ross's lab have completed a set of studies of the influence of nuclear localization on huntingtin toxicity. Constructs with an export signal had decreased toxicity, while constructs with an NLS had increased toxicity, in transient transfection assays using N2a neuroblastoma cells.

While evidence on the involvement of protein degradation pathways in HD remain circumstantial at present, future studies on this topic promise to shed important light on the cellular mechanisms that are important in the pathogenesis of HD.

5) Cell culture and peripheral cell models of HD.

Leslie Thompson and David Housman described experiments using PC12 cells expressing huntingtin under the control of an ecdysone-regulated promoter. After inducing the expression of expanded huntingtin, approximately 10% of these cells develop nuclear inclusions. These cell lines can be induced to differentiate in the presence of NGF, and are therefore attractive models to investigate mechanisms of cellular pathogenesis in neuronal cells.

A number of other cell lines have been used to study polyglutamine diseases; these include human 293 cells, COS7 cell, CB1 monkey cells, and VP16 cells. Steve Suhr reported on subtle but reproducible deleterious effects of expressing polyglutamine proteins in such cells. In general, expression of polyglutamine proteins seem to increase the sensitivity of cells to the toxic effects of sub-lethal doses of apoptosis-inducing agents such as tamoxifen. One observation from these cells is that cell division may be necessary for the expression of the toxic effects of polyglutamine proteins. This conclusion is based on the observation that if the cells are grown to confluence, they arrest their growth. These cells acquire inclusion bodies, but are relatively insensitive to stressors such as tamoxifen. If the cells are

split, they begin to divide, and then die. There is some suggestion that this may also reflect a defect in cytokinesis.

There were several suggestions for generating new cell lines that might be useful in investigating HD. Ethan Singer raised the question of whether primary neurons could be made to express both telomerase to get cells past crisis and SV40 large T antigen to prevent senescence. Michael Greenberg responded that this sort of thing has been tried in the past with other oncogenes, but the results have been frustrating and not generally useful for studying differentiated properties of neurons. Along similar lines, Allan Tobin suggested embryonic stem cells as a source of differentiated neuronal cultures. This approach is probably feasible, but is not likely to be easy or rapid, and would require a major effort and long-term commitment. Dr. Tobin reported on another approach--the use of AAV (adeno-associated virus) delivery of GFP-huntingtin constructs into the striatum. After 5 days there was GFP staining present in the brain, with many cytoplasmic aggregates; after 12 days loss of cells was observed. Although there was increased TUNEL staining in sections, there was no co-localization of TUNEL staining with aggregates, suggesting that the aggregates were not necessary for apoptosis. After 6 months, there were a few remaining aggregates; viral expression of the huntingtin constructs was probably turned off by this time.

Cell culture systems promise to provide answers to the cellular mechanisms responsible for pathogenesis in HD, and such systems seem well worth pursuing and expanding. In particular, it would be extremely useful if new molecular or cellular markers for disease progression, other than cell death, could be identified.

6) Additional markers for the progression of HD.

To date, there has been relatively little information on the changes that occur in neurons expressing expanded huntingtin, other than intranuclear inclusions and cell death itself. Clearly, the identification of additional markers would be tremendously useful, both for understanding the mechanism responsible for development of the disease as well as for monitoring the progression of the disease, and evaluating treatments. Functional parameters, such as electrical excitability, release of neurotransmitters, energy metabolism, Ca^{2+} regulation, and synaptic stability are aspects of neuronal function that should be examined. Marie-Francoise Chesselet mentioned NMDA-induced cell swelling as a marker for the disease in brain slices of HD mice. Michael Greenberg suggested that it might be valuable to examine the effects of growth factors to see if there are changes in the phosphorylation of channels that could underlie neuronal dysfunction.

Akria Sawa, working with Chris Ross, observed that there are changes in mitochondrial function in cells of HD patients. Peripheral lymphocytes from patients with juvenile-onset disease are more sensitive to apoptotic agents such as staurosporine. Inhibitors of the mitochondrial permeability transition such as cyclosporin block this increased sensitivity. Quantitative MRI may be useful to diagnose and monitor disease progression, since some volumetric analyses (carried out by Elizabeth Aylward) have recently shown that shrinkage of the caudate/putamen precedes diagnosable HD. In addition, there are reports of hypermetabolism in the occipital cortex presymptomatically.

There is a major interest in the possibility of using gene screening to analyze possible markers for disease. Chip and filter technologies offer the possibility of

screening for large numbers (>20,000) of genes. Despite a consensus that such technology offers exciting opportunities, there was considerable caution expressed about the practicality of obtaining meaningful results, and the difficulty of eliminating many spurious changes and false positives. There was general agreement that such efforts should be carefully planned, involve group discussion and participation, and recruitment of industry participation if possible. This would be a productive topic for a future workshop.

Table 1. Transgenic mouse strains available for the study of polyglutamine diseases.

Lab	# CAG	Transgene size	Back ground	Promoter	Expression level	Behavior	Pathology	Inclusions
HD								
Tagle	(16), 48, 89	full-length	FVB-N	CMV/ SV40 enhancer	All lines ~5X_ (except one line)	Heterozygotes: 8wk foot-clasping 12wk vertical pole defect 32wk hyperactivity: circling, _ exploration 60wk hypoactivity 68wk death Homoz: earlier (except clasping) Sx onset are 4-8 wk earlier than full-length transgenics, except foot- clasp (at 8wk)	48: none 89: at hypoactive stage: degeneration, TUNEL ⁺ & GFAP ⁺ in stri, ctx, hippoc, thal 20% cell loss	89: starting at ~12wk, in stri, ctx, Purkinje cells
	(16), 48, 89	1-171aa (exons 1-3)	FVB-N	CMV/ SV40 enhancer	lower than full, higher than endogenous			
Ross/ Borchelt	(18), 44, 82	1-171 aa (exons 1-3)	C3H/BL6	Prion	Protein: 1/5- 1/10† RNA: 10X_	82 only (several lines): 8wk weight loss 12wk hypoactivity 22wk rotarod defect 28wk wasting, uncoordination, tremor, death	82: at 28 wk have neuritic deposits (huntingtin ⁺), swollen neurites in ctx & SN, neuronal degeneration	82: ~18 wk ctx, stri, hippoc, cereb
Mandel	73	full-length	BL6/DBA	CMV, no enhancer	1/10†	Line was lost		
MacDon ald	(18), 48, 90, 109	knock-in	129/CD1	endog	endog	48: circling in 1% of mice 90, 109: at 1yr nothing obvious slight polyQ-dependent instability, male > female	48: at 2yr no pathology, no gliosis, no TUNEL ⁺	48: none at 2yr
Myers	72, 80	knock-in	FVB- N/BL6	endog	Protein: ~2X†	Heteroz: hyperactivity, aggression Homoz: not (?) mild polyQ instability	at 2yr no pathology at 2-3mo heteroz have synaptic fn defect: † paired pulse facilitation	at 2yr Ub ⁺ , in wild type too

Lab	# CAG	Transgene size	Back ground	Promoter	Expression level	Behavior	Pathology	Inclusions
HD								
Bates	130-150	exon 1	C57Bl6/[CVA]	human HD	~endogenous	tremor	inclusions in <u>all</u> brain nuclei	staining depends on Ab used: 1) most~4 weeks 2) EM48 Ab ~3 wks 3) ubiquitin in inclusions at ~5 wks
Hayden	(18), 46, 72	YAC	FVB-N	endog	protein: 46: _ endog 72: _ endog and 2X endog	46: 3 lines, homozygous and heterozygous. at 20-24 mo: no obvious behavior phenotype. 72: 2 lines. low expressor: no behavior at 8 mo. 72 high expressor had hyperactivity and circling at 6 wk, did not transmit.	46: at 6 mo progressive \uparrow LTP; at 20-24 mo diffuse nuclear stain (no NI), no degeneration. 72 low and high expressors: at 12 mo had degeneration in lateral striatum.	72: no in low expressor, yes in high expressor

Lab	# CAG	Transgene size	Back ground	Promoter	Expression level	Behavior	Pathology	Inclusions
AR								
Merry	(16), 112	109aa+Q (no ligand, DNA, NLS and transactiv domains)	BL6/SJL	Prion	present, level not known. copy# 2-10	2 founders & F1. 3 wk: gait (stiff hind), tremor, hypoact, _grooming, seizures, rotarod defect, weight loss 2founders. 3-4wk: hyperact, circling, 3founders: no symptoms 3founders. 4-5mo: claspng	one F1 dead at 7wk no obvious cell death	in most neurons. anterior horn: multiple NI, single in Purkinje most Ub ⁺ Hsc70 ⁺ , few Hsp70 ⁺ , some Hsp40 ⁺ 20Score ⁺ and 19S ⁺ , no 11S ⁺
DRPLA								
Ross/Borchelt	(26), 65	full-length	C3H/BI6	Prion	prot & RNA: endog & lower	65Q (several lines): 6-8wk tremor, seizures, ataxic gait, rotarod 4mo-1yr death	intense nuclear stain	NI and nuclear stain 65Q: 125kD fragment in dentate, stri, ctx, cereb; expression no cleavage in stem & olf bulb

Key to abbreviations: SN= subthalamic nucleus, cx=cortex, stri= striatum, cereb= cerebellum, fn=function, Ub⁺=ubiquitin positive staining, LTP= long-term pothentiation. endog=endogenous, prot=protein, olf bulb=olfactory bulb, Q=polyglutamine, NLS= nuclear localization sequence