

**Innovation and Standardization: Accelerating the  
Search for Huntington's Disease Therapies**  
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## **Abstract**

Over the past decade, a wide array of approaches and tools has been recruited in the quest to understand and, ultimately, cure Huntington's disease (HD). Yet still much about HD's progression and scope remains obscure. At the "Innovation and Standardization: Accelerating the Search for Huntington's Disease Therapies" workshop, participants probed HD's complexities and generated new proposals for tackling the disease. An important topic was the circuitry of the basal ganglia and how these connections might be altered in HD. Participants noted that, although much research has been directed at understanding the vulnerability of striatal medium spiny cells, many other brain regions and cell types are affected. In particular, the importance of cortical alterations was highlighted, and the potential roles of interneurons and glial cells were discussed. Participants agreed that cells which are not visibly damaged, but are dysfunctional, might be at the root of some of the disease's major pathologies. HD was analyzed as a disease involving both cell autonomous processes and disruptions in cell-cell interactions.

Participants also discussed the role of mutant huntingtin in development, as well as the possibility that activity levels or metabolic load of different cells and neural pathways may contribute to their vulnerability. Compensatory mechanisms were also examined, and ideas to understand these in greater depth were proposed. Looking ahead, participants stressed the need to move beyond correlations and identify the causal relationships that link many of the alterations described in HD. They suggested tools to facilitate this goal, including advances in mouse genetic engineering, new cell lines, the use of two-photon imaging and uncaging techniques, and computer modeling of basal ganglia circuitry.

Efforts to develop therapies for treating HD were analyzed as well. Rational approaches with defined targets—including the use of siRNA to knock down mutant huntingtin, intrabodies, and small molecule blockers of aggregation—were discussed, as well as screens using model organisms to reverse specific readouts. Inhibitors of histone deacetylases and of huntingtin proteolysis were examined, as well as the use of combination therapies. The direction of future efforts in HD therapeutic development was a topic of intense discussion. Some participants favored focusing on HD's primary target, mutant huntingtin, while others considered that understanding basal ganglia circuitry and its disease-associated alterations would likely be critical for understanding HD and finding a cure.

## **Introduction**

One of the most striking features of Huntington's disease is its multiple faces. Although the disease has its roots in a single, well-defined dominant mutation—a CAG expansion in the huntingtin gene—its consequences have proved complex and multi-faceted. HD victims experience, not only motor disruptions, but cognitive and emotional symptoms which can arise early during the progression of the disease.

Illustrating how HD touches upon many aspects of a person's health, a 31-year old woman suffering from HD and her husband generously shared their experiences with workshop participants. The young woman, who once excelled at various intellectual and athletic pursuits, is now unable to walk without assistance, can barely speak, and has trouble organizing her thoughts and remembering things. She must take medication to control her seizures, dystonia, and myoclonus. At times, noted her husband, she is obsessive and aggressive. Although the woman is now at an advanced stage of HD, even in the early stages of disease her health was affected in

multiple ways. At first, her gait became subtly disrupted, and she had difficulties drafting in her architecture classes. In addition to these motor symptoms, she had trouble concentrating, as well as organizing and completing her class assignments.

The young couple's heartbreaking situation made a strong impression on workshop participants. Participants were inspired to help develop strategies to understand the biology of HD and accelerate the search for a cure. They were also made keenly aware that HD is not limited to one class of symptoms, and that its devastating effects are probably the result, not only of the failing of individual cells, but of alterations in interactions between cells. Indeed, a major focus of the workshop was the circuitry of the basal ganglia and how these connections might be altered in HD.

## **Basal Ganglia Circuitry**

A large fraction of HD research has focused on understanding the toxic effects of mutant huntingtin on medium spiny cells (MSNs), the striatal cells which express the most extensive signs of measurable damage. Yet, as noted by Carl Johnson, the relative importance of this visible damage has actually not been clearly established. It is possible, for example, that cells that send inputs to the MSNs, such as cortical neurons or striatal interneurons, are the primary sources of dysfunction, and that MSNs are simply downstream victims. Glial cells may also be key players in the pathological process. And as revealed by new anatomical studies, even cells that are not directly associated with the striatum may be importantly involved in the disease process given that alterations in many brain areas have been detected early on.

### ***Circuitry overview***

Ann Graybiel provided participants a starting point for examining these issues by presenting an overview of basal ganglia circuitry based on anatomical studies and receptor distributions (Fig. 1). The striatum is the main input structure of the basal ganglia, receiving excitatory afferents from the cerebral cortex and thalamus. Its main output is the internal globus pallidus (GPi), and ultimately, the thalamus. The circuitry of the basal ganglia has been proposed to mediate several functions. For example, some investigators have suggested that the globus pallidus is where programs from the cortex are selected for execution. Others have postulated that the striatum is where decisions on future actions are made. Still others have proposed that the basal ganglia provide a circuit for making reward-based decisions and that, once a learned pattern is established, it is stored in the cortex or the brainstem. In general, the striatum seems to be important for learning and executing repetitive tasks.

The striatum can be divided into two broad compartments, the striosomes and the matrix. In addition, projection neurons can be differentiated by the neuropeptides they contain, their projection targets, and the dopamine receptors they express (dopamine regulates glutamate input in 5-8% of the spines of spiny projection neurons). Most striatal neurons are GABAergic spiny projection neurons (77% to 98%). The remaining neurons are interneurons, classified as cholinergic, or GABAergic containing either parvalbumin or calretinin, or neuropeptide Y, somatostatin and NADPH diaphorase. As noted by Charles Wilson, medium spiny neurons receive several thousands of excitatory glutamatergic synapses and hundreds of inhibitory GABAergic inputs from interneurons. Recent studies indicate that, although much fewer in number, the interneuron synapses exert strong effects, modulating the timing of spiny neuron firing (Tepper et al., 2004).

The nature of cortico-striatal projections was of particular interest to participants given that many studies have implicated these connections in HD pathology. Graybiel explained that input from the frontal cortex is topographic, such that projections from the motor cortex preferentially target the putamen. In addition, the mapping is somatotopic. For example, movement of the foot seems to activate the dorsal putamen, while face movements activate more ventral and medial regions of the putamen. Saccadic eye movements induce bilateral activation at the junction between the body and the head of the caudate nucleus and in the right putamen (Gerardin et al, 2003). Other cortical regions also send inputs to the striatum, including the parietal and temporal cortices.

Pointing out new observations, Graybiel noted that the pedunculopontine nucleus (PPN) in the reticular formation, has recently gained prominence in basal ganglia circuitry. The basal ganglia are more highly interconnected with the PPN than with any other brain region. The PPN appears to provide an interface for the basal ganglia to affect sleep and waking, and both structures play roles in learning and reward-based behavior (Mena-Segovia et al, 2004). Graybiel also noted the existence of a pathway from the cortex to the subthalamic nucleus (STN) known as the hyperdirect pathway. In addition, she noted the increasing relevance of backprojections (e.g., from the GP to the striatum, and from the thalamus to the striatum). As noted by Wilson, the external GP (GPe) may importantly affect striatal output through its projections onto striatal interneurons. Another area of ongoing research is the influence of basal ganglia on cortico-thalamic pathways. Graybiel noted that the thalamus and cortex have very tight loops connecting them, such that projections from the thalamus map to a very restricted area in the cortex which in turn projects to the corresponding small area in the thalamus. The basal ganglia project to the thalamus in these areas, but the function of these projections has remained elusive.

The complexity of basal ganglia circuitry is not only due to the multiplicity of anatomical connections, however. As Wilson explained, many of the neurons of the basal ganglia are oscillators, characterized by regular spontaneous firing in the absence of any input. *In vivo*, however, none of the neurons express their regular firing patterns because they are modulated by multiple inputs. The result is that it is very difficult to predict how any given perturbation will affect the circuit. Intuition alone is insufficient and often leads to incorrect conclusions.

Anton Reiner proposed using computational simulations to better understand and predict the behaviors of such complex circuitry. He suggested using these simulations to perform virtual experiments in which one or more components are altered, and then compare the output with data from real experiments. Graybiel noted that several researchers are working on computational models of basal ganglia function and that it might be worth contacting them. In addition, Stephen Tapscott drew a parallel between basal ganglia circuitry and genetic networks, and wondered whether similar strategies could be applied to analyze basal ganglia.

### ***Implications for HD***

As noted by Anne Young, the disruption of any component in the circuit can lead to a severely distorted output resulting in dysfunctional movement and learning. Indeed, HD patients often have trouble executing new movements on command, while still being able to perform fairly complicated patterns of movement that have already been learned (e.g., tying a shoelace). Diana Rosas added that the striata of healthy controls, but not those of presymptomatic HD patients, display large waves of activity during the early learning of a motor task.

Exactly how the basal ganglia's pattern of activity is distorted in HD patients, however, is unknown. As noted by Wilson, this is a major open question in HD research whose answer could

help focus experiments enormously. Wilson added that in Parkinson's disease, long-term electrode implantations have provided relevant information, but comparable data are not available for HD. It is not even clear whether HD-altered output is more or less harmful than having no output at all. Jim Olson asked if anything was known about how the activity of basal ganglia circuits changes during sleep, a time when HD patients do not experience choreic movements.

Nancy Wexler proposed exploring the possibility of implanting electrodes in volunteers with HD, even if they do not serve a therapeutic purpose as in Parkinson's disease. In addition, participants suggested using several HD models to record neural activity in awake, behaving animals. A monkey model of HD is being developed, noted Flint Beal, as well as a sheep model, noted Johnson. Marie-Françoise Chesselet added that currently available transgenic rat models may also prove useful.

The recording technology used to monitor activity is also an important issue to consider. Marina Chicurel noted that high density recordings might provide an informative view of basal ganglia circuits by exposing the simultaneous activities of a representative sample of cells in a local circuit. György Buzsáki at Rutgers University has used micromachining and lithography to create silicon probes sporting 96 recording sites, each separated from its neighbors by only 100 to 300 micrometers. Besides allowing high density recording, the slender probes cause less tissue damage per recording site than other electrodes. Graybiel, who has been recording local field potentials in cortex and striatum during task performance, said her team is interested in using these probes.

Graybiel also noted that it would be very helpful to have a means of distinguishing between substance P-containing spiny cells and enkephalinergic spiny cells in these types of electrophysiological experiments. As recently reported by Deng and colleagues (2004), the striatal projection to the GPe made by enkephalinergic spiny cells is the most vulnerable in HD, showing substantial loss by grade I. In contrast loss of the projections to the GPi made by substance P-containing spiny cells proceeds more gradually.

Participants also stressed the need to look beyond the striatal medium spiny cells for HD-related abnormalities. Wilson noted that a lesson to be learned from Parkinson's disease is to avoid overfocusing on the cells that, at first blush, appear to be the most affected. In Parkinson's disease, researchers initially concentrated on the substantia nigra pars compacta (SNc) but have since discovered more fruitful areas of research in the SNc's targets.

### ***Looking beyond the striatum***

Participants agreed that it was time to look beyond the striatal spiny cells for clues to HD pathology. As noted by Rosas, who is conducting morphometric and volumetric analyses of human MRI images, widespread degeneration of the brain occurs in HD, even in the early to mid-stages of disease. Subjects with HD have significant volume reductions in almost all brain structures, including total cerebrum, total white matter, cerebral cortex, caudate, putamen, globus pallidus, amygdala, hippocampus, brainstem, and cerebellum (Rosas et al, 2003). Rosas suggested that some of the clinical symptoms of HD, and perhaps their heterogeneity, may be related to this widespread degeneration.

Evidence for extrastriatal effects of HD in the brain was also provided by Alex Osmand who has developed a technique to detect aggregation foci (AF) in postmortem tissues. AF are sites that can actively recruit monomeric polyglutamine molecules. The results reveal much heterogeneity between human HD and animal models, as well as between models. Nevertheless,

they all indicate that AF are present early in HD progression, in many different brain regions, including the cortex, thalamus, SNc, olfactory tubercle, nucleus accumbens, and several midbrain nuclei. Of particular interest, the presence of AF in the cortex, striatum, and thalamus, suggests that the cortico-striatal-thalamic loop may be affected by mutant huntingtin at every step.

### ***Looking beyond the striatum: The cortex***

Several participants cited evidence implicating the cortex as a potential site of primary dysfunction in HD. Indeed, Michael Levine, who has conducted extensive electrophysiological studies in various HD mouse models, said his group is shifting its focus from the striatum to the cortex because of increasing evidence that cortical function is disrupted early on. Levine has found that, at very early stages of the disease, striatal spiny cells display spontaneous depolarizations that emanate from the cortex. A similar effect can be seen in normal animals when the cortex is made hyperexcitable using bicuculline to block GABA receptors. Interestingly, striatal neurons expressing dopamine D2 receptors (i.e., enkephalinergic spiny cells) are the most affected. However, the HD-induced hyperresponsiveness is transient. At approximately 5 weeks of age, the striata of R6/2 mice start showing progressive reductions in spontaneous excitatory currents, as if the cortex and striatum have been disconnected. Spine density and synaptic markers, including PSD-95 and synaptophysin, decrease. In the YAC, Aronin and R6/2 models of HD, more current is needed to activate striatal cells when stimulating the cortex. Levine suspects that, as the striatal cells lose their glutamatergic inputs, their GABAergic inputs begin to dominate. Edward Stern, who has been recording cortical cells *in vivo* in R6/2 mice, said his results were consistent with Levine's findings. He added that he has observed increased firing of cortical neurons in response to current injection.

Participants also described microscopic evidence of early cortical pathology. Marion DiFiglia reported that in a new mouse model of HD developed by Neil Aronin which develops pathology and symptoms similar to those of R6/2 mice, but over a longer time scale, there is a strong correlation between the onset of motor deficits and cortical pathology. Chesselet cautioned, however, that cortical involvement is variable between HD models, and there are cases in which motor deficits and striatal pathology are observed without any overt sign of cortical damage.

In humans, there is evidence that cortical alterations appear early on. Osmand has found AFs in large pyramidal neurons, predominantly in layers III and V/VI. In rare cases, he has seen them in bipolar neurons and interneurons. Based on the intracellular distribution of foci in pyramidal neurons, Osmand hypothesizes that AF accumulate and grow within the cytoplasm, migrate to the axonal pole, and then into the proximal regions of axons and sometimes dendrites, ultimately giving rise to neuropil aggregates which do not recruit additional poly-glutamine molecules. He thinks it is unlikely that AF correspond to the postulated neurotoxic oligomeric species suggested by others (e.g., Charles Glabe and colleagues) because AF appear to persist for relatively long periods. Nevertheless, AF may affect cellular function, for example by sequestering polyglutamine-containing proteins.

In addition, Rosas described regionally specific cortical damage detectable by MRI at early stages of HD. Even before disease onset, she has observed subtle changes in the cortex. One of the clearest alterations is cortical thinning of the superior part of the sensori-motor gyri. The thinning is more pronounced in some areas than others. For example, regions projecting to the tail of the caudate, corresponding to representations of the feet, seemed to be particularly

affected. Interestingly, problems with foot movements are often reported in the early stages of HD. Regional differences in the cortex have also been detected at the transcriptional level. Jim Olson noted that microarray analyses indicate that Brodmann areas 4 and 9 of the cortex are differentially affected. Area 4, which corresponds to the primary motor cortex and projects to the striatum, is the most affected cortical area. Its transcriptional alterations share the most similarities with the caudate, the brain area with the highest number and greatest magnitude of expression changes.

Rosas also monitored fractional anisotropy to assess white matter integrity. She noted early changes in the white matter underneath the motor cortex, and in the posterior limb of the internal capsule. Young pointed out that this might explain the hyper-reflexivity which is observed relatively early in HD patients. In addition, Rosas noted a correlation between subjects' performance on cognitive tests—including the Unified Huntington's Disease Rating Scale (UHDRS) and Stroop tests—and their degree of cortical, but not striatal, degeneration. Rosas is now extending her studies to include earlier time points. Her observations, so far, derive from patients who already have some degree of striatal atrophy (these patients have no motor symptoms, however, perhaps due to compensatory mechanisms).

Participants were enthusiastic about Rosas's results. William Yang, who has generated mice that express mutant huntingtin in specific cell populations, noted that cortical pyramidal neurons and their axons and dendrites are relatively easy to monitor in mice and hopes to do so in future experiments. Beal noted that abnormal burst firing in local regions of the cortex has been detected in HD patients using magneto-encephalography. Very low-level electrical changes have been detected even in pre-symptomatic patients. And Olson wondered whether the changes in cortical thickness observed by Rosas might be useful as indicators of disease progression in clinical trials.

The process by which a deficit in the cortex might lead to striatal damage was also discussed. Reiner noted that there are two main types of corticostriatal neurons: those that project only intratelencephalically (IT-type) and have small terminals, and those that send their main axons into pyramidal tracts with a collateral projection to the striatum (PT-type) and which have larger terminals (Lei et al, 2004). Interestingly, the PT-type neurons project to the enkephalinergic spiny neurons, while the IT-type neurons project to substance P-containing neurons which are affected later in HD progression. Reiner suggested that the larger PT-type terminals may contribute to the increased vulnerability of enkephalinergic cells. Rosas noted that her results are consistent with this model, and Wilson added that the firing of PT-type, but not IT-type, neurons correlates with movements.

In addition, Young mentioned her group is exploring the mechanisms of cortically-mediated excitotoxicity. They are now repeating an experiment originally performed in wildtype mice in which animals became insensitive to excitotoxic drugs after decortication. Interestingly, these animals regained their sensitivity when drug exposure was coupled to metabotropic receptor stimulation.

### ***Looking beyond the striatum: Other brain regions***

Participants also discussed the involvement of other brain areas in HD. Osmand noted that he observes AF in the mediodorsal thalamus and SNc very early on in most animal models of HD. In addition, he has observed early AF in the neurites of olfactory tubercle and nucleus accumbens neurons in a transgenic rat model of HD which displays social anxiety and increased

vocalizations at an early age. Rosas has also seen damage in the accumbens in humans, but only in later stages of disease. Beal suggested that adrenergic input may be protective.

Regions involved in controlling eye movements were also discussed. DiFiglia pointed out that the interface between the white and gray matters in the striatum, a region involved in eye movement control, was greatly affected in HD. Osmand added that in two mouse models, AF are found in several components of the oculomotor pathway, including the trochlear and abducens nuclei and in tegmental regions of the pons. If AF cause functional alterations in these cells, this may help explain the early oculomotor deficits reported in human HD. Osmand also wondered whether dysfunction in one nucleus could trigger a cascade of pathological changes along the whole pathway.

Cha noted that eye movements are one of the best indicators of basal ganglia function. Olson suggested using eye movements to monitor drug efficacy. Eye movements could help physicians fine-tune the dosages of currently used medications, which are hard to optimize because of patient variability. They could also be used as a biomarker in clinical trials. Indeed, Young wondered whether monitoring eye movements could have helped pre-select patients in a recent tetrabenazine clinical trial in which there was significant inter-patient variability.

Another brain region that appears to be affected by HD is the corpus callosum. Rosas has preliminary results indicating a regional gradient of changes in this structure. In presymptomatic patients, she has seen alterations in the posterior aspect of the corpus callosum trunk and is now performing a more detailed analysis of shape changes all along the structure. She plans to correlate these alterations with her observations in cortex.

Participants also discussed the potential involvement of the cerebellum. The cerebellum projects in the same general vicinity of the thalamus as the basal ganglia (the motor thalamus), and some researchers have proposed that these projections may overlap or intercalate. However, there appeared to be no clear evidence for early cerebellar involvement in HD. Rosas said it was difficult to image this region of the brain, and Beal added that his group has observed changes in cerebellar white matter associated with HD, but they are late.

Mary Kennedy wondered whether activity levels may contribute to a pathway's vulnerability to HD. She suggested the existence of a "use it *and* lose it" phenomenon, in which very active pathways might be disrupted earlier than less active ones. This could help explain the observed variability in symptoms between patients. Consistent with this possibility, Rosas noted that the left side of patients' brains is usually more affected than the right side. Jang-Ho Cha pointed out that patients often report early symptoms that interfere with their occupations (e.g., a roofer may complain about losing his balance, while an accountant may describe having difficulty concentrating), but it is difficult to discern whether these symptoms are reported early because occupation-related symptoms actually develop sooner, or because they are noticed earlier. As suggested by Beal, it might be informative to examine the results from PET imaging studies that have been performed to track metabolic changes in presymptomatic patients.

Olson added that metabolic demand might be a key factor determining how different brain regions and different cells respond to mutant huntingtin. He suggested that AF, for example, may present a metabolic load that could eventually overwhelm cells and lead to both cell and pathway dysfunction. One way to test the importance of metabolic demand, Olson proposed, is to block one eye of a HD animal and assess whether it reduces pathology in its associated neural pathways due to the resulting reduction in metabolic rate.

## **Cell and molecular mechanisms of disease**

### ***Ion channels and neurotransmitter receptors***

Chesselet wondered whether examining the circuitry problem from a bottom-up perspective might complement studies that directly characterize circuit dysfunction. In particular, she suggested looking at whether changes in gene expression—especially of ion channels and neurotransmitter receptors—could help illuminate changes in circuitry. Olson noted that his group has identified transcriptional changes in calcium signaling genes, but has detected no major changes in ion channel or neurotransmitter receptor genes.

Nevertheless, post-transcriptional changes in these proteins, or localized transcriptional alterations undetectable by whole tissue analyses, may be very important in HD pathology. Working with acutely dissociated striatal cells from R6/2 mice, Levine has identified a subpopulation of neurons that is hyperresponsive to NMDA as early as 15 days after birth, a time when dendritic spines are still developing. Studies using RT-PCR indicate that, at 21 days, NMDA receptor NR2A subunits are decreased, which may imply that NR2B subunits are increased. The findings are particularly intriguing in light of Lynn Raymond's observations implicating NR2B subunits in the vulnerability of striatal spiny cells.

Kennedy noted that these results may be evidence of a very early damage to the synaptic developmental program. In normal animals, NR2B subunits are present during embryogenesis and are gradually replaced by NR2A subunits. If this normal progression is disrupted in HD, then pathology may start very early in life. Reiner noted that studies from Marcy MacDonald's group were consistent with mutant huntingtin having developmental effects. MacDonald observed developmental defects in mice expressing reduced levels of huntingtin, and a rapid neurological disease if the mice also expressed mutant huntingtin.

To determine to what extent synaptic development is disrupted, Kennedy suggested examining other synaptic molecules, such as CaM kinase II and SynGAP. In addition, Olson and Johnson suggested analyzing the Yamamoto mouse model of HD, in which expression of mutant huntingtin can be switched off at different stages of development.

There are some complexities associated with the neurotransmitter receptor results, however. Levine noted that Marjorie Ariano has found decreases in NR2A, as well as NR2B, subunits in individual cells of symptomatic R6/2 striata, while NR1 subunits are increased. Yet Cha noted he observed no changes in the expression of NMDA receptor subunits in the striata of 1-year-old YAC128 mice, and very minor alterations in the striata of R6/2 mice, as assessed by *in situ* hybridization and receptor binding. Levine added that he has observed reduced NMDA synaptic responses in 6 month-old YAC128 mice. These apparently conflicting results may be explained by differences between model systems, changes in the distributions of synaptic and extrasynaptic receptors and, perhaps most importantly, the timing of the experiments. Many HD-associated alterations change dramatically with age. Accordingly, Levine plans to perform age-series experiments.

The electrophysiological behavior of HD cortical and striatal neurons, however, is surprisingly normal. Stern noted that even in 12 week-old R6/2 mice, spontaneous cortical activity looks strikingly similar to that of control mice. And although Levine reported decreased potassium inward rectifier and delayed rectifier currents in spiny striatal cells from several HD mouse models, Stern said that at 8 weeks of age the striatal "up" and "down" states of R6/2 mice appear normal.

Levine agreed that the electrophysiological behavior of HD neurons is remarkably similar to that of normal neurons. However, he also noted that if there were dramatic changes, the

disease would probably be fatal at a very early age. DiFiglia and Chesselet said that alterations could be important but subtle (e.g., in the ratios of NMDA receptor subunits). Stern agreed and clarified that he observes small, but significant alterations in cortical cell responses associated with HD. Another consideration pointed out by Yang is that the anesthesia Stern uses in his experiments may mask some cortical defects. Graybiel agreed and added that it was crucial to select the proper anesthetic to avoid artefacts. Finally, Wilson urged participants to expand their studies to include downstream neurons in motor pathways. If no clear alterations are observed at a particular level, it may be that the primary pathology is actually occurring downstream.

### ***Transcriptional disruptions***

Several studies have shown that transcriptional alterations are associated with HD. As described by Olson, recent results from the HD Human Array Group indicate that in postmortem brains with relatively low-grade HD pathology, the caudate has the highest number and greatest magnitude of expression changes. Intracellular signaling genes are significantly disrupted, while genes involved in apoptosis, oxidative stress, and protein folding do not seem to be substantially affected. In addition, as previously mentioned, there are regional differences in the cortex which may shed light on the underpinnings of selective vulnerability. For example, it is possible that transcriptional deficits make some cells less capable of responding to stress than others.

A particularly intriguing transcriptional alteration was described by Elena Cattaneo. She and her colleagues found that the expression of several genes involved in cholesterol synthesis is decreased in cell and animal models of HD, as well as in the human HD brain. In R6/2 mice, the decrease is observed in the cortex and striatum, even in mice that are only 4 weeks old. Cholesterol is of particular interest because it is produced locally in the brain and is required for synaptic remodeling. Interestingly, all of the affected genes are regulated by the SREBP transcription factor, and an SRE-Lac reporter construct confirmed that cells expressing mutant huntingtin are impaired in SREBP-mediated transcription. Moreover, localization studies revealed that SREBP's translocation to the nucleus is impaired in HD cells, and cholesterol administration rescued cultured striatal cells from death in a dose-dependent manner.

Participants were intrigued by these results and suggested several ideas to extend the work. Kennedy noted that Edoardo Marcora in her lab is using hippocampal cells to study the translocation of transcription factors from the synapse to the nucleus, and she suggested using this system to probe SREBP's translocation in normal and mutant cells. In addition, Greg Lemke proposed crossing R6/2 mice with transgenic mice that overproduce cholesterol. Cattaneo was concerned, however, that such mice may have secondary alterations that could interfere with the interpretation of the results. She wondered whether a mouse that overproduces cholesterol exclusively in the brain exists. Beal suggested providing extra cholesterol in the diet, as has been done in experiments studying Alzheimer's disease.

Participants also suggested ideas for analyzing HD patients. Rosas noted that her HD patients seem to have an unusually low incidence of heart disease, and Cha said none of his patients is taking statins. It might be informative to look at HD medical records to assess whether these trends are more widespread. In addition, Chicurel suggested measuring 24S-hydroxycholesterol, a cholesterol derivative produced by neurons that crosses the blood brain barrier, in serum and/or cerebrospinal fluid of HD patients. Previous studies have shown significantly higher levels of this compound in Alzheimer's disease and vascular demented patients at early stages of disease. Beal added that measuring cholesten, a cholesterol oxide, might also be informative.

### ***Quality control mechanisms***

To address the question of vulnerability, Jeffery Kelly suggested taking a closer look at the quality control mechanisms of different cells. In particular, he proposed examining the distribution of chaperones, as his group has done for several amyloid diseases. He noted that the chaperone composition of different cells seems to correlate strikingly well with disease vulnerability. Gillian Bates said she has investigated chaperone function in HD, but the story is complicated. Indeed, some efforts to link chaperone function with the amelioration of HD pathology have yielded negative results. For example, both Bates and Beal have crossed HD mice with Hsp70 transgenic mice, but Beal observed no changes in pathology and Bates noted only a one week delay in aggregate formation and no change in the behavioral phenotype. As it may also be necessary to upregulate Hsp70 co-chaperones, Bates continues to be interested in compounds that affect the heat shock response and their potential therapeutic effects. In addition, Yang noted that his group is testing the effects of chaperones as genetic modifiers of HD.

Participants also discussed mutant huntingtin turnover. Peter Waldmeier suggested that an examination of this rate may provide important clues about HD pathology. DiFiglia noted that these experiments are difficult to do, but there is some evidence indicating that wildtype huntingtin has a relatively slow turnover rate, and mutant huntingtin fragments have even slower rates. The Yamamoto mouse model, in which aggregates disappear when mutant huntingtin is turned off, might provide a useful system to extend these observations.

### ***Cell-autonomous processes and cell-cell interactions***

Regardless of the specific molecules and cellular processes involved in HD, a fundamental question is whether pathology is caused by a cell autonomous process in which mutant huntingtin disrupts cells individually, or whether mutant huntingtin affects interactions between cells, or whether both processes contribute to pathology. To address this question, Yang used the Cre/LoxP system to generate mice that express a neuropathogenic form of mutant huntingtin (mHtt-exon 1) in specific cell populations. He has initially studied mice that express mutant huntingtin in all neurons in the brain (pan-neuronal model), or only in the vulnerable cortical projection neurons (cortical model).

Although all models develop aggregates, only the pan-neuronal model shows motor deficits, early and robust reactive gliosis, abnormal neuronal processes called dysmorphic neurites, and emergence of dark neuron degeneration. Thus, Yang's results suggest that some aspects of HD pathology, such as aggregate formation, are cell autonomous. However, most of HD's key deficits—progressive motor symptoms, gliosis, dysmorphic neurites, and dark neuron degeneration—depend importantly on cell-cell interactions. As described by Chesselet, HD can be thought of as a combination of two diseases: a cell autonomous disease and a circuit disease.

Some open questions remain, however. As pointed out by Cha, one possible interpretation of Yang's results is that the pan-neuronal model has a more severe phenotype simply because it expresses more mutant huntingtin in the entire brain. In addition, it remains unclear how mutant huntingtin causes cell-autonomous and cell-cell interaction effects, and how selectivity occurs. As noted by Yang, mutant huntingtin may exert certain types of toxicities that are common to different cell types, it may also exert other types of toxicities that are specific to a given cell type, and it is crucial for us to understand both types of toxicities in order to design optimal therapeutic strategies. A new mouse model developed by Yang should help address these questions. Yang has generated mice that express full-length human huntingtin with a 103

polyglutamine repeat from a Bacterial Artificial Chromosome (BAC), in which mutant huntingtin can be turned off selectively in different cell types.

### ***Looking beyond the medium spiny cell: Interneurons***

Until now, HD research has mostly focused on the striatal medium spiny cell because it is the cell that is most obviously affected by the disease. However, participants presented evidence implicating other cell types as potentially key players. In particular, interneurons emerged as likely contributors to the disease process. Wilson noted that medium spiny cells receive very strong inputs from striatal interneurons. Graybiel added that strong connections exist between the cortex and the parvalbuminergic interneurons in the striatum, which seem to provide widespread inhibition in a center-surround fashion and may help filter cortical inputs. Consistent with this, Levine suggested that, as striatal cells in R6/2 and R6/1 mice lose their glutamatergic inputs, GABAergic inputs, probably from interneurons, begin to have more influence on spiny neuron behavior. And if these inputs are abnormal, they could have dramatic effects on the basal ganglia's output.

The altered function of striatal interneurons may result from a cell-autonomous process and/or cell-cell interactions. For example, if cortical interneurons are dysfunctional, this may lead to disrupted cortical inputs to both striatal spiny cells and striatal interneurons, which together may cause spiny neuron dysfunction. Levine is interested in doing time course experiments to determine how hyperexcitability changes as HD progresses. To examine the role of interneurons in greater depth, he is now crossing R6/2 mice with mice that express GFP in their interneurons.

In addition, Yang reported that, while spontaneous excitatory input to cortical pyramidal neurons is normal in his pan-neuronal model, the spontaneous inhibitory input to these pyramidal neurons from cortical interneurons is altered early on. His working hypothesis is that interneuron dysfunction causes cortical pyramidal cells to become hyperactive which, in turn, causes excitatory toxicity to the striatal medium spiny cells. Yang will extend these findings using his new BAC model to specifically turn off mutant huntingtin in cortical interneurons. He will also use mouse lines that express GFP in interneurons to study their morphology and electrophysiology.

Participants were excited about the potential role of interneurons in HD and suggested additional experiments to extend Levine's and Yang's observations. For example, Cha proposed studying striatal spiny cells and interneurons in co-cultures, and Young suggested examining EEG tracings for signs of cortical interneuron dysfunction. In addition, Cha and Chesselet proposed behavioral studies to examine the possibility that HD patients' difficulty in shifting tasks might be explained by a disruption of suppression mechanisms mediated by interneurons.

Participants also discussed some of the findings' therapeutic implications. Graybiel wondered whether increasing GABA in the cortex might be beneficial and Young asked whether a combination of valproate, an anti-epileptic drug which increases GABA levels, and lamotrigine, an anti-epileptic drug which affects sodium and calcium channels, might have therapeutic value.

The interneuron findings also induced participants to re-think the roots of HD pathology. As noted by Beal, whereas striatal spiny cells are lost in HD, striatal and cortical interneurons are mostly spared. Thus, as suggested by Johnson, the cells that are lost in HD may not be the true villains, but rather, those that survive and malfunction.

### ***Looking beyond the medium spiny cell: Glia***

DiFiglia noted that another cell type that is rarely discussed, but may importantly contribute to HD pathology is the glial cell. In the striatum, reactive astrogliosis coincides with cell loss. In addition, reactive microglia, which release cytokines, appear in the cortex and in the striatum where cell loss is first apparent, in a grade-dependent manner.

In addition, Chicurel noted that Paolo Guidetti has evidence implicating microglia in HD pathology. Guidetti has observed that the excitotoxin quinolonic acid and its bioprecursor, 3-hydroxykynurenine (3-HK), both produced by microglia, are elevated in the neostriatum and neocortex of HD patients at early stages of disease. In addition, a genetic screen carried out by Flaviano Giorgini to identify loss-of-function suppressors of mutant huntingtin toxicity identified kynurenine 3-hydroxylase, the enzyme that generates 3-HK. Cattaneo's studies indicating an HD-associated disruption in cholesterol synthesis may also involve glial cells, since astrocytes seem to supply neurons with most of their cholesterol requirements.

### ***Looking beyond the medium spiny cell: Cells outside the brain***

Participants also noted that several cell types throughout the body seem to be affected by HD. As noted by Olson, Andy Strand has found that the activities of genes associated with fast-twitch muscle function—e.g., glycolysis—are decreased in HD, while those involved in slow-twitch function—e.g., lipid catabolism—are increased. Strand hypothesizes that HD may drive a transition from fast to slow muscle fiber types. In addition, significant changes in gene expression appear to occur in kidney, liver, and fat cells.

### ***Compensatory mechanisms***

Despite all these observed alterations, some of which may begin very early in life, the onset of HD symptoms is characteristically late. Participants agreed that understanding the homeostatic mechanisms that help organisms cope with mutant huntingtin expression could be key to understanding HD progression. As pointed out by Nancy Wexler, it has been very difficult to identify HD's primary disruptions and isolate the effects of compensatory processes, yet understanding these processes might yield valuable therapeutic leads. Following up on Kennedy's suggestion that HD might cause early developmental alterations, participants proposed that organisms expressing mutant huntingtin may be able to initially compensate for these alterations and function normally. But, as described by Carl Leventhal, they eventually express the disease when their compensatory mechanisms break down.

Yang suggested examining whether the "one-hit model" of neurodegeneration applies to HD (Clarke et al, 2001). If HD causes cumulative damage in individual neurons and there is a threshold at which compensatory mechanisms are overwhelmed and start failing, then the probability of neuronal death should increase with age. On the other hand, if a single catastrophic intracellular biochemical event, analogous to radioactive decay, leads to neuronal death—as proposed in the one-hit model—then the kinetics of cell death should be exponential. In this case, the risk of death is constant, death occurs randomly in time, and the death of each neuron is independent of other neurons. By examining the kinetics of cell death, Clarke and colleagues (2001) have found that several neurodegenerative diseases, including HD and Parkinson's disease, appear to fit the one-hit model. Applying similar analyses to HD may help illuminate the disease process and the role of compensatory mechanisms.

There are many possible compensatory mechanisms that may be relevant to HD. One intriguing possibility was described by Cha. In R6/2 mice, Cha observed decreases in the NMDA

receptor subunits NR2A and NR2B in the hippocampus, and slightly in the cortex, using in situ hybridization and receptor binding techniques. Cha hypothesized that the hippocampus might be less vulnerable to huntingtin toxicity because of this early down-regulation of NMDA receptors. Interestingly, Osmand has never seen AF in the hippocampus.

Participants also discussed associated therapeutic implications. Olson noted that if the body is already doing its best to compensate for mutant huntingtin's damage, then targeting the primary cause of the disease might be the most productive approach, rather than attempting to bolster homeostatic mechanisms. On the other hand, as noted by Leventhal, there may be opportunities for improving endogenous compensatory processes. If so, therapeutic interventions such as motor training or tissue transplantation may help alleviate symptoms and/or slow down their progression.

### ***Looking ahead: Global strategies***

As noted by Lemke, a major problem in understanding HD and distinguishing compensatory mechanisms from primary pathology is that most of the data available are strictly correlative. Lemke noted that HD research has generated a very long list of alterations associated with HD, but the causal links remain unknown. For example, the correlations identified by microarray scans include causal relationships, but they also include epiphenomena, and statistical noise. The causal information can only be revealed through further hypothesis-driven experimentation, which has yet to be widely applied. A related challenge, pointed out by Cha, is identifying the phenotypes that are most relevant to HD, distinguishing those that are directly linked to the primary pathology from those that are compensatory or epiphenomena.

Participants agreed that genetic systems are likely to be particularly useful for addressing this problem. Young reminded participants of *Drosophila*'s strengths, and Yang described powerful mouse genetic tools. He pointed out that the Gene Trap Consortium has a large collection of knock-out mice, and the BAC transgenic approach to overexpressing genes in mice can be readily scaled up. In addition, he noted that applying "gene therapy" techniques in mice, by crossing gene overexpression or loss-of-function mutants of candidate genes with a robust mouse model of HD will allow one to screen for candidate genetic modifiers of HD. Such genetic modifier experiments could be used as a first-pass validation of candidate pathways that are relevant to pathogenesis and treatment of HD. In many cases, Yang noted, such experiments could be performed by a small group of researchers, using relatively small numbers of mice.

Participants also highlighted the potential benefits of applying what has been learned about other neurodegenerative diseases to the study of HD. The circuitry-based approaches used to study Parkinson's disease and the resulting data were considered particularly valuable. In addition, Tapscott wondered whether a Huntington's disease-like 2 (HDL2) mouse model exists and, if so, whether studying its electrophysiology could shed light on HD mechanisms. Leslie Thompson noted that, together with Larry Marsh, their group is currently making a HDL2 fly. Also, Tapscott suggested looking more carefully into the disease mechanisms of other CAG diseases.

Evolutionary analyses of the sequences and functions of huntingtin orthologues was suggested as yet another strategy to illuminate HD pathology. Cattaneo noted that her team is using an evolutionary bioinformatics approach to gain insights into huntingtin's normal function. Their working hypothesis is that huntingtin has an ancestral function, probably related to development, and over the course of evolution, it gained a second, neuronal-specific function, perhaps serving as a synaptic scaffold. To examine this hypothesis in greater depth, Cattaneo is

cloning huntingtin from a few key species whose huntingtin genes have not been sequenced yet. In the future, she plans to do complementation assays in flies and mice.

Cattaneo also noted that *Drosophila* huntingtin is quite different from that of humans—it is larger and has neither polyglutamine nor polyproline regions. Cattaneo asked whether this dissimilarity, coupled to *Drosophila*'s very different brain circuitry, may be an important limitation in its use as an HD model system. Lemke noted that inter-species variability in poly-amino acid stretches, such as polyglutamine and polyproline, may not be very relevant because these regions have very low levels of conservation and are usually thought to be unstructured. Also, Johnson cautioned that analyzing huntingtin evolution was not a straightforward task. For example, huntingtin's distribution across the phylogenetic tree is unexpected: slime molds have a huntingtin orthologue, yet *Caenorhabditis elegans*, which has a well-developed nervous system, does not. Thompson added that their *Drosophila* model expresses human mutant huntingtin and, when this expression is pan-neuronal, it probably affects neuronal circuitry because alterations are found in several areas of the nervous system, including major brain structures such as the mushroom bodies.

### ***Looking ahead: New tools***

Participants also discussed new tools that promise to importantly accelerate HD research. Adam Carter described a two-photon imaging (TPI) system for the study of calcium dynamics in striatal slices. The system makes use of two-photon laser scanning microscopy (TPLSM) and two-photon laser uncaging (TPLU). Carter pointed out that TPLSM is less harmful to cells and can penetrate more deeply than confocal microscopy. Moreover, TPLU has the same advantages as TPLSM, and can be used to excite small subcellular regions using caged compounds. Working in Bernardo Sabatini's lab, Carter has used this technique to uncage glutamate and mimic miniature EPSCs at single dendritic spines. He can use whole-cell recordings to hold the cells at different potentials and monitor the EPSCs while tracking changes in calcium concentrations at individual spines using calcium-sensitive dyes.

In a recent publication using these techniques, Carter reports that the contribution of L-type calcium channels increases, while that of T-type channels decreases, in the dendrites and dendritic spines of medium spiny cells when they transition to the "up" state (Carter and Sabatini, 2004). In addition, the primary source of synaptic calcium switches from AMPA receptors to NMDA receptors. Carter is now assessing the roles of metabotropic receptors 1 and 5, and plans to examine the contributions of different NMDA receptor subunits. His preliminary results indicate that NMDA responses are sensitive to ifenprodil, an NR2B blocker. Carter is also interested in using TPLSM and TPLU to study the ways in which cortico-striatal inputs control MSN firing.

To tackle HD, Carter is setting up a lentiviral slice model of HD. In addition to probing medium spiny cell physiology in this model, he is interested in examining interneurons and glia. Chicurel suggested using Reinhart and Lo's system which uses biolistics to deposit beads carrying huntingtin constructs into neurons in organotypic brain slices. Transfection and inclusion formation can be easily monitored because the huntingtin constructs, as well as the beads, carry fluorescent tags. In addition, co-transfection of multiple genes is easily performed by loading multiple DNA constructs onto single beads. Also, expression levels can be titrated because they correlate well with the amount of DNA loaded onto the beads. However, Carter said that while they are interested in this complementary approach, they prefer to use *in vivo* injections and acute slices because organotypic slices can develop anomalous circuits.

Another set of powerful tools discussed at the workshop were cell model systems. The ST14A cell line, derived from embryonic day 14 rat striatal primordia by retroviral transduction of the temperature-sensitive SV40 large T antigen, for example, has many of the characteristics of striatal medium spiny cells. Cattaneo said that ST14A cells expressing a knocked-in mutant huntingtin construct provide a very stable and robust cellular model of HD. In addition, her lab is working with immortalized, heterozygous and homozygous, knock-in lines derived from Marcy MacDonald's mice, as well as a huntingtin inducible cell line. Immortalized cell lines from Venezuelan HD patients are also available, noted Wexler. Of particular interest, Johnson noted that Lynn Raymond and others have primary striatal cells that are hypersensitive to glutamate which promise to allow high throughput testing of various therapeutic candidates.

The use of stem cells for HD research was also discussed. Cattaneo noted she has homogenous neural stem cells derived from embryonic mice which can be expanded as a monolayer in serum-free media, as well as differentiated *in vitro* into glia or GABAergic neurons. The cells are stable, and have not been immortalized. Similar cells, said Cattaneo, can be obtained from humans. Beal added that stem cells engineered to express telomerase have been used to experimentally treat spinal cord injury with some success. In addition, his group is working with cells that can differentiate into dopaminergic neurons, and which might be able to differentiate into GABAergic neurons.

Wexler also pointed out that the collection of Venezuelan HD tissues is now being managed by Jean Paul Vonsattel at Columbia University. She noted that the organizational efforts had resulted in some delays, but the tissues should now be readily available and she urged participants to use this resource. In response to Olson's and Bates' suggestion to get more non-brain tissues, Wexler noted that the Columbia tissue bank actually has a variety of such samples.

## **Therapies**

Johnson presented a brief overview of ongoing efforts to develop therapies for treating HD. He described rational approaches with defined targets, as well as screens using model organisms to reverse specific readouts. He noted that the Hereditary Disease Foundation's role in these efforts is to help identify screens and assays to find and test primary candidates. The subsequent process of turning leads into drugs will be handled by pharmaceutical companies.

### ***siRNA***

Johnson noted that major recent advances in the siRNA arena include improvements in the design and delivery of these molecules. Researchers have discovered that most of the specificity of 22 nucleotide-long siRNAs lies in the first 8-10 nucleotides. In addition, designing siRNAs with a mismatch at the 5' end is now known to enhance knockdown success rates. siRNA introduced into cells interacts with cellular proteins to form the RNA induced silencing complex (RISC). Before, or at an early stage of, RISC formation, a helicase preferentially unwinds siRNA duplexes at the less stable end. An siRNA strand with a less stable 5' end is incorporated into the RISC more efficiently than the opposite strand. If this strand is the antisense strand, then it will induce target mRNA degradation more effectively. Using these and other guidelines, it is now possible to make effective siRNAs for virtually any sequence. However, even efficient siRNAs do not knock down expression completely. Participants

described 30-70% efficiencies in various in vivo studies. Johnson noted that 95% efficiency has been achieved using hyperfunctional siRNAs in cell culture.

As noted by Wexler, however, the efficiency, as well as the allele specificity, required for achieving beneficial effects in HD is unknown. Johnson added that a related, unresolved question is: what are the effects of knocking down normal huntingtin in an adult? Chesselet stressed that knockdown phenotypes should be examined very carefully because subtle changes, which may result in tragic consequences, could be easily overlooked. Levine agreed and noted that neuronal dysfunction, rather than cell death, should be monitored. Examining the effects of knocking down huntingtin in higher animals was also suggested. Johnson said such experiments were underway in monkeys.

Regarding delivery, Johnson noted that, so far, adeno-associated viral systems have proved more effective than lentiviral systems. In addition, “gutless” adenovirus, which lack parts of the viral genome and can thus carry large amounts of heterologous DNA, have been required for some applications. Stefan Kochanek, for example, has used these vectors to deliver a shRNA-resistant copy of wildtype huntingtin along with the therapeutic shRNA, which does not distinguish between endogenous species of mutant and normal huntingtin mRNAs.

Rainer Kuhn suggested using cannuli and pumps to continuously deliver siRNA directly into the brain. Kuhn also noted that lipid leaflets known as cochleates are useful for enhancing delivery. Cochleates have a hydrophobic interior and a multi-layer “wrapped” configuration. They directly adsorb to cells’ outer membranes and deliver their cargo to the cells’ interior.

Chesselet asked how delivery could be targeted to multiple brain regions, given that HD pathology seems to be so widespread. Johnson acknowledged this was an important consideration and said that viruses could be injected at multiple sites or, as suggested by Beal, into the cerebrospinal fluid. In addition, Johnson noted that there are mechanisms to enhance viral spreading.

### ***Intrabodies***

Paul Patterson gave a brief summary of his lab’s efforts to develop intrabodies, single chain Fv antibody fragments, against mutant huntingtin. He noted that certain intrabodies against epitopes that flank the polyglutamine region decrease aggregation and promote survival in cultured cells and flies. Although intrabodies’ mechanism of action is unknown, it is speculated that they either alter the kinetics of mutant huntingtin misfolding or interfere with mutant huntingtin’s access to other cellular components.

Patterson’s group is advancing the development of intrabodies in several ways. They are currently re-engineering a particularly effective intrabody to increase its stability, and they are beginning to test intrabodies in mice using viral vectors. In addition, working with Ron Wetzel, Patterson has generated antibodies against polyglutamine filaments with a range of antigenic preferences—some are generic for amyloid structures, while others bind more specifically to poly-glutamine stretches or to particular amyloid proteins.

Patterson is also setting up a new screening system to identify novel intrabodies that enhance cell survival by transfecting PC12 cells expressing an inducible mutant huntingtin construct with an intrabody library from a non-immunized animal. Olson and Yang suggested Patterson switch to a striatal model system, using either isolated cells or brain slices. However, Patterson noted that the PC12 system is advantageous because mutant huntingtin expression can be regulated, and initial screens can be carried out with low levels of mutant protein. In addition,

as previously discussed, it is unclear whether striatal cells are the most, or only, relevant cell type.

### ***Small molecules***

Several small molecule screening efforts are currently underway. As described by Johnson and Young, readouts include aggregation inhibition, toxicity inhibition in model organisms, inhibition of huntingtin cleavage, enhancement of huntingtin clearance, and regulation of the expression of other genes (e.g., dopamine D2 receptor expression).

A particularly advanced effort, summarized by Young, is the discovery and development of C2-8, a potent inhibitor of polyglutamine aggregation now in preclinical trials in mice (R6/2 and 140 knock-in mouse). C2-8 was discovered by Kazantsev and colleagues (2005) using a yeast-based high throughput screening assay to identify compounds that inhibit polyglutamine aggregation. The assay revealed four promising hits which were validated in mammalian cultured cells, structurally optimized for potency, and tested in organotypic brain slices derived from HD mice. As explained by Bates, the slice assays involved monitoring aggregates using an automated imaging system to detect fluorescently labeled aggregates. Although the total number of aggregates was similar in HD transgenic vehicle-treated slices and HD slices treated with C2-8, the treated slices had aggregates with smaller areas. The slice assay also allowed the researchers to test the compounds' effects over several weeks. C2-8 emerged as a particularly potent anti-aggregation molecule, with inhibitory effects detectable at 10 nM, and long-term inhibitory effects. In addition, it was shown to suppress neurodegeneration *in vivo* in *Drosophila*. As noted by Young, C2-8 can be administered orally and it crosses the blood-brain barrier quite readily. In addition, toxicity assays performed by Novartis so far indicate that C2-8 appears to be safe at concentrations that inhibit aggregation.

C2-8's mechanism of action is not yet understood, however. Young said that experiments using labelled C2-8 to observe the compound's location in the cell are currently underway. In addition, Chicurel suggested testing C2-8's effects on various huntingtin aggregate forms distinguishable by different antibodies. Kuhn cautioned that it is very difficult to prove that an antibody recognizes a particular configuration. However, Johnson noted that Glabe's antibody, which recognizes toxic soluble oligomers, has been well characterized. Indeed, Thompson is using this antibody to examine the effects of C2-8 in cell lysates and filter assays. Beal added that gel laddering techniques could also be used, as has been done in Alzheimer's disease.

Another promising set of small molecules which decrease neurodegeneration in flies and mice are histone deacetylase (HDAC) inhibitors. As noted by Thompson, SAHA and phenylbutyrate, which appear to be safe and tolerable in clinical trials, are particularly promising candidates. Thompson is now examining these compounds' mechanisms of action using Biacor technology to study protein-ligand interactions, as well as expression profiling.

Studies of HDAC inhibitors have also underscored the potential benefits of using combination therapies. As noted by Thompson, Larry Marsh genetically reduced each of the three different classes of HDACs and found that decreasing classes I and III, but not II, was effective in alleviating HD toxicity. Interestingly, the simultaneous reduction in classes I and III resulted in a synergistic effect. Thus, it is possible that therapies could be developed in which two or more inhibitors are used simultaneously at low doses to achieve better clinical outcomes, while minimizing undesirable secondary effects. Indeed, combination cocktails including other compounds besides HDAC inhibitors have been tested with encouraging results. As described by Thompson, combining SAHA with cystamine, a compound which acts upon multiple targets, and

Congo Red, an aggregation blocker, yielded dramatic effects when each compound was used at low concentrations which had no effects when used singly. Another example is a cocktail including SAHA, geldanamycin, and a ROCK inhibitor. As noted by Young, the synergy observed in these cases is much greater than additive.

However, Kuhn noted that such combinations were rarely of practical use because of the difficulty of performing the required toxicity tests. Each compound has to be approved separately, as well as in combination—a process which can be very costly and time-consuming. Nevertheless, participants considered that the information obtained from these experiments could be useful for the development of new therapies.

Small molecule screens are also generating promising candidates for enhancing huntingtin clearance and preventing proteolysis. Young said small molecules that selectively clear mutant huntingtin have been identified, and proteasome screens are underway. In addition, DiFiglia noted that her group has developed a high throughput screening assay to identify compounds from a focused library of small molecules that inhibit mutant huntingtin proteolysis. The team is specifically monitoring the production of putative aspartyl protease fragments Cp-A, which appears to be key to the formation of nuclear inclusions, and Cp-B, which appears to be involved in cytoplasmic aggregate formation, using Western blots. In addition, DiFiglia is collaborating with Scott Zeitlin to study the effects of deleting huntingtin's putative aspartyl protease site in transgenic mice.

Participants discussed the advantages and limitations of this approach. Johnson considered that, before investing more effort, it might be useful to first identify the protease and confirm its role in mutant huntingtin proteolysis. Kuhn cautioned that much effort has been invested in finding inhibitors of aspartyl proteases, with little success. This may be because aspartyl proteases' catalytic sites are very large and non-specific. He wondered whether intrabodies against aspartyl proteases might be worth testing. However, DiFiglia noted that the small molecules she has identified do not necessarily act upon the protease's catalytic site. For example, they may instead interfere with upstream regulatory processes.

Kuhn noted that it is important to have a defined target because in the drug discovery process there are many optimization steps that depend on this knowledge, including the chemical optimization of efficacy and specificity, and the minimization of toxicity. He urged participants to think about how clinical trials will be done and what quantitative measures will be used to assess candidate therapies' effects. Several participants acknowledged Kuhn's concerns, but considered that promising leads should not be automatically discarded if they lack a defined target. They argued that, even if they are not optimal, these leads may provide valuable therapeutic possibilities. Indeed, Young reminded participants that the targets of the large majority of drugs on the market today remain unknown.

### ***Looking ahead: Global therapeutic strategies***

The direction of future efforts in HD therapeutic development was a topic of intense discussion. Kuhn argued that the major strength of HD is its defined genetic mutation. Thus, he considered that future efforts should focus on mutant huntingtin, rather than on downstream targets. Lemke observed that understanding the circuitry alterations in HD will likely illuminate basic mechanisms of the disease process, but agreed with Kuhn that, from a therapeutic standpoint, it makes sense to focus on the obvious target: mutant huntingtin. Young added that she liked the idea of modeling basal ganglia circuitry, but like Lemke, acknowledged it probably shouldn't be a priority for therapy development. She added that one therapeutic benefit of

studying basal ganglia circuitry, and how it's disrupted in HD, would be the development of a deep-brain stimulator (e.g., to be placed in the GPI). However, this would be directed at developing a symptomatic treatment, rather than a curative therapy.

On the other hand, several participants considered that basal ganglia circuitry was of prime importance for the understanding and design of rational treatments for HD. Chesselet reiterated that HD can be thought of as two diseases: a cell-autonomous process, which probably progresses slowly, and a circuit disease, which may participate in a vicious cycle with developmental disruptions (e.g., in synaptic and ion channel functions). Thus, focusing exclusively on cell-autonomous biochemical alterations is unlikely to be sufficient to understand and therapeutically solve the disease. Johnson added that therapies directed at correcting circuitry disruptions may not only help alleviate symptoms, but may possibly slow down disease progression, given that cell-cell interactions are crucial for the expression of the disease.

As pointed out by Cha, no one disagrees that approaches to directly target mutant huntingtin, such as siRNA, should be pursued. The question is: to what extent should other approaches be examined? While some participants, such as Kuhn, considered that nearly all efforts should be focused on this well-defined target, others felt that considerable attention should be paid to cell-cell interactions and circuitry issues.

A related question discussed by participants was how much effort should be invested in approaches that promise to yield therapeutic opportunities in the short-term, versus approaches which promise to illuminate basic disease mechanisms, but whose therapeutic benefits may take longer to materialize. Most participants agreed that both strategies should be pursued. Wilson noted that long-term approaches are often the most fruitful, and Waldmeier agreed, noting that short routes to therapeutic solutions are usually overly optimistic. Johnson considered that about 20% of resources should be allocated to quick routes.

## **Action items**

### Basal ganglia circuitry

- \*Build computer models of basal ganglia circuitry (Reiner) and contact researchers involved in these efforts (Graybiel).
- \*Apply genetic network algorithms to the study of basal ganglia circuitry (Tapscott)
- \*Examine how circuit activity changes during sleep (Olson)
- \*Perform recordings in awake, behaving rats, monkeys, and sheep (Chesselet, Beal, Johnson)
- \*Use Buzsaki's high-density recording electrodes (Chicurel, Graybiel)
- \*Monitor basal ganglia activity in HD human volunteers (Wexler)
- \*Extend MRI and PET studies of HD pre-symptomatic patients (Rosas, Beal)
- \*Examine pyramidal cortical neuron electrophysiological behavior in Yang's transgenic mouse models (Yang)
- \*Test effects of mGluR stimulation on excitotoxicity sensitivity of decorticated animals (Young)
- \*Examine correlations between corpus callosum alterations and cortical alterations in MRI images (Rosas)
- \*Explore feasibility of "use it and lose it" proposal of HD pathology (Kennedy)
- \*Test the importance of metabolic demand by blocking one eye of a HD animal, and comparing the pathology of its associated neural pathways to those of the open eye (Olson)
- \*Examine striatal electrophysiology in greater depth in Stern's in vivo system (Wilson, Stern)
- \*Monitor how striatal hyperexcitability changes over time (Levine)

### Cellular mechanisms

- \*Extend observations of NR2A and NR2B subunit expression during development and assess the expression of other synaptic molecules, e.g. CaM kinase II and SynGAP (Levine, Kennedy)
- \*Use Yamamoto's inducible HD model to examine HD developmental defects (Olson, Johnson)
- \*To extend Cattaneo's studies of cholesterol alterations:
  - a) Probe SRBP's translocation in normal and mutant hippocampal cells (Kennedy)
  - b) Cross R6/2 mice with cholesterol-overproducing mice (Lemke) or administer extra cholesterol in the diet (Beal)
  - c) Examine incidence of heart disease and statin use in HD medical records (Rosas, Cha)
  - d) Monitor plasma and/or cerebrospinal fluid levels of 24S-hydroxycholesterol and cholesterol in HD patients (Chicurel, Beal)
- \*Evaluate chaperone distribution and composition in different cell types (Kelly) and test the effects of chaperone modifiers (Yang)
- \*Examine mutant huntingtin turnover (Waldmeier)
- \*Test whether the "one-hit model" of neurodegeneration applies to HD (Yang)
- \*Use two-photon imaging and uncaging techniques to assess the roles of metabotropic receptors, and different NMDA receptor subunits in medium spiny cell physiology (Carter)

### Dissecting the roles of different cell types

- \*Analyze BAC mouse generated by Yang turning off mutant huntingtin in various cell types (e.g., cortical pyramidal neurons, interneurons, glia) (Yang).
- \*Monitor interneuron function by crossing R6/2 mice with mice that express GFP in their interneurons (Levine)
- \*Study striatal spiny cells and interneurons in co-cultures (Cha)
- \*Examine EEG tracings in HD patients for signs of interneuron dysfunction (Young)
- \*Conduct behavioral studies to explore the connection between HD patients' difficulty shifting tasks and the possible disruption of suppression mechanisms (Cha and Chesselet)
- \*Use in vivo lentivirus injections to express mutant huntingtin in medium spiny cells and probe the physiology of these cells, as well as that of interneurons and glia using two-photon imaging and uncaging techniques (Carter)
- \*Collect more non-brain HD tissues (Olson, Bates, Wexler)

### Global strategies

- \*Direct more effort toward identifying causal links, rather than just correlations (Lemke)
- \*Use mouse genetics to validate candidate genetic suppressor pathways for HD using BAC-mediated transgenesis (Yang)
- \*Apply lessons learned from other diseases (Parkinson's disease, CAG diseases, HDL-2)
- \*Extend studies on huntingtin evolution—clone huntingtin from key species whose huntingtin genes have not been sequenced yet, do complementation assays in flies and mice (Cattaneo).
- \*Take advantage of multiple cell lines available (ST14A cells, neural stem cells) (Cattaneo, Beal, Wexler)
- \*Keep therapy research focused on how clinical trials will be done and what quantitative measures will be used to assess candidate therapies' effects (Kuhn)

## Therapies

\*Examine whether regulating GABAergic transmission has therapeutic potential (e.g., valproate and lamotrigine) (Graybiel, Young)

\*RNAi therapy:

- a) evaluate the effects of knocking down wildtype huntingtin in adults using mice (Yang), and higher animals (Johnson)
- b) use cannulae and pumps to continuously deliver siRNA directly into the brain (Kuhn)
- c) use cochleates to enhance siRNA delivery (Kuhn)

\*Intrabodies:

- a) Engineer more stable intrabodies and test them in mice using viral vectors (Patterson)
- b) Extend studies of antibodies against protofibrils (Patterson, Wetzel)
- c) Screen intrabody library from non-immunized animal in PC12 model (Patterson)

\*Probe C2-8's mode of action by tracking labelled C2-8 in cells (Young), and testing C2-8's effects on various huntingtin aggregates using conformation-specific antibodies (Chicurel, Thompson) and gel laddering techniques (Beal)

\*Examine HDAC inhibitors' mode of action using Biacor technology and expression profiling (Thompson)

\*Continue evaluating combination therapies (Thompson, Young), but keep in mind difficulties involved in human trials (Kuhn)

\*Aspartyl protease inhibitors

- a) Identify relevant aspartyl protease and confirm its role in huntingtin proteolysis (Johnson)
- b) Analyze hits from screen for inhibitors of the generation of Cp-A and Cp-B huntingtin fragments (DiFiglia)
- c) Assess effects of deleting huntingtin's putative aspartyl protease site in transgenic mice (DiFiglia, Zeitlin)
- d) Test effects of intrabodies against aspartyl proteases (Kuhn)

## Biomarkers

\*Assess whether cortical thinning observed by Rosas might serve as a clinical biomarker (Olson).

\*Evaluate alterations in eye movements as potential biomarkers for clinical trials and for fine-tuning medication dosages (Olson)

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