

# Hereditary Disease Foundation

## Phenotype Assessment in Mouse Models of Huntington's Disease

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Prepared by Marina Chicurel

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In some ways, a cure for Huntington's disease (HD) seems close at hand. We know that HD is a disease of the basal ganglia and cortex, and even more precisely, that the medium spiny cells of the striatum are particularly affected. Huntingtin, the single gene underlying the disease, has been cloned, and its defect -- stretches of CAG repeats -- pinpointed precisely. In addition, animal models have been created that, to varying degrees, reproduce the devastating symptoms and neuropathology characteristic of HD patients. Given the talented group of molecular biologists, cell biologists, anatomists, and physiologists supported by the Hereditary Disease Foundation (HDF), one might predict that HD is well on its way towards becoming a treatable disease.

As is so often the case in biology, however, complexity has a way of humbling our predictions. There is indeed good reason for hope and excitement in HD research, but several problems are yet to be solved. It is still unclear, for example, how accurately current animal models mirror human disease. And drawing general conclusions from the models presents a significant challenge since each model is unique in its manifestations of symptoms and pathology.

It is also far from clear why only a few cells, such as the spiny cells of the striatum, become ravaged by disease when huntingtin is expressed throughout the body. Focusing on the spiny cells may seem like the best way to tackle this puzzle, yet increasing evidence suggests that much could be missed by studying spiny cells in isolation. Spiny cells form part of complex neuronal circuits whose ensemble behaviors may play important roles in disease progression. Factors released by cells in these circuits, including neurotransmitters and growth factors, could be critical. Finally, the temporal sequence of events that marks the progression of disease is still uncertain. The causal associations that link cell loss, cellular inclusions and abnormal behaviors remain a mystery.

These issues and their ramifications were discussed in the workshop "Phenotype

Assessment in Mouse Models of Huntington's Disease." By generously sharing her experiences with the group, a person with Huntington's disease helped focus the discussion on a search for knowledge leading to potential therapies. Many and varied insights emerged. Most importantly, participants generated a valuable list of experiments that promise to move the scientific community one step closer to finding a cure for HD.

### What should a mouse with HD look like?

#### **Challenges and unresolved issues**

Everyone agrees that a good animal model is one that reproduces the essential features of human disease. But reaching a consensus on what these features are can be a challenging task. Allan Tobin recounted how, after a long and painful discussion a few years ago, researchers at an HDF workshop agreed that their criterion for a good mouse model for HD would be one with a loss of enkephalinergic neurons that project from the striatum to the globus pallidus (GP). Even today, after the generation of several different mouse models, however, the point is still a subject of debate.

As summarized by Peggy Shelbourne and shown in Table 1 on page 16, HD mouse models vary widely. They differ in the size of their transgene (ranging from 48-repeat knock-ins to YAC chromosomes carrying the full-length huntingtin gene), the size of the CAG repeats they carry (ranging from 44 to 150), the promoters that drive the transgene (ranging from the endogenous huntingtin promoter to potent CMV and SV40 promoters), the site of integration of the transgene into the genome, and the genetic background of the mice.

It is hardly surprising, then, that the pathology and behavior of these mice is far from uniform. More dramatic symptoms are expressed in mice carrying exon I of the huntingtin gene than the full-length copy, for example. And as has long been appreciated, longer CAG repeats tend to result in an earlier onset of disease. The location and levels of transgene expression vary according to the choice of promoter and to the site of transgene integration. Finally, different strains of mice vary in their wildtype brain anatomy, physiology, and behavioral patterns.

Shelbourne was particularly concerned about this latter source of variation. She described significant variation between mice strains and even substrains. For example, she pointed out how the responses of different strains to neurotrauma, such as administration of kainic acid, vary widely. Ann Graybiel mentioned the large variability between the size and shape of the corpus callosum in different mice strains. Even more potentially confounding, Shelbourne noted that behaviors that are accentuated in HD mice, such as aggression, back-flips, and compulsive behaviors, are also widely variable between strains. In addition, a comprehensive and standardized battery of assays to test the different models is still lacking.

### **Potential solutions**

Participants made several suggestions to deal with these problems. Many agreed that because each model has its unique strengths and weaknesses, future studies should employ several models for comparison. Shelbourne stressed the importance of appropriate control selection to deal with strain variability and recommended a recent review by Oswald Steward and colleagues (*Exp Neurol* [1999] 157:19-42). To help define what HD "looks like in a mouse," John Marshall suggested using traditional lesions and pharmacology. Whereas these types of studies have been done in primates and rats, very little is known about mice, particularly different strains of them. Marshall proposed a broad-brush study in which he could use the expression of immediate early genes to monitor the effects of dopamine agonists and antagonists in the striatum and GP. To administer the compounds, he proposed using a relatively non-invasive cortical well placed over the dura of the mice. The approach would provide a read-out of events beyond the initial responses of neurons and a functional survey of the distinct neuronal populations in these brain regions. In addition, since much information can be obtained using small numbers of mice, the approach could provide a screen for the vulnerability of particular neurons to the huntingtin mutation.

### ***Behavior as a marker of HD***

Since the models have yet to provide clear associations between cell loss, the presence of inclusions, and abnormal behavior, Charles Wilson proposed using behavior -- the ultimate expression of

disease -- as the main criterion for choosing models for electrophysiological studies. Tobin, however, warned that behavior is very difficult to analyze. Many different disruptions can lead to altered brain function. Thus, dissecting which alterations are HD-specific can be complicated. Shelbourne agreed, mentioning that most commonly used tests such as foot-clasping, beam walking, and the rotarod are generally too coarse to capture early deficits. In addition, the tests are very stressful. Poor performance can be due to a decreased ability of coping with stress, rather than true motor dysfunction.

Shelbourne's group is developing new assays -- that identify subtle abnormalities in gait, for example -- which better capture HD-relevant behaviors at earlier ages. Interestingly, her preliminary results suggest that one of the earliest behavioral manifestations of HD in her knock-in mice is increased aggression. This characteristic is not observed in other HD mice. Marshall suggested examining other behaviors as well, such as alternating tasks and habit formation. Regardless of how behavior is measured, however, Marie-Francoise Chesselet stressed that behavior is an end-point, and that electrophysiological studies should probably be carried out well before mice express altered behaviors.

### ***Beyond mice: Rat and primate models of HD***

Given these problems, and even more importantly, the lack of baseline knowledge and experimental systems designed for mice (including stereotaxic surgery and chronic implantation of cannulae or recording devices), participants discussed the feasibility of developing rat and primate models of HD. Hagai Bergman, for example, stressed that development of a primate model was important since the basal ganglia of rodents and primates differ substantially. Although a few supported the idea of developing transgenic rats and/or primates, most participants agreed with Tobin's suggestion of using viral vectors instead. The advantage of using viruses to introduce mutated genes is that it is much less technically demanding and time-consuming than producing transgenics. The disadvantage, however, is that only a subset of cells express the mutant protein.

### ***Looking beyond the difficulties of mice: What can they tell us?***

Although generating these new models appealed to some, several participants stressed the need to accommodate their experiments to the existing mouse models. James Surmeier said that for reductionist experiments like his examining ion channels and signaling proteins, generalizing across strains and even species was often not a problem. For those doing less reductionist work, Michael Levine encouraged participants to characterize and compare the physiology of the various HD mice. He stressed that this kind of exploratory work was required for laying the foundations.

Levine's laboratory has performed electrophysiological experiments in brain slices of R6/2 Bates mice, between 60 and 110 days old, using both standard sharp electrodes and whole cell voltage clamp with patch electrodes and infrared videomicroscopy for cellular identification to study the behavior of the medium spiny cells. His findings (Levine et al., *J. Neurosci. Res.* 58: 515-532, 1999) revealed several intriguing abnormalities. The HD cells were approximately 10 mV more depolarized than control cells, and had wider action potentials and greater input resistances. Wilson and Surmeier suggested that these characteristics might be explained by a decrease in inward rectification. Inward rectification is mediated by potassium channels that open in response to hyperpolarization and induce further hyperpolarization. As the channels open, they increase the cell's leakiness, thus decreasing the input resistance. Blocking these channels results in depolarization and increased input resistance. Levine agreed with this possibility, but thought the full explanation was probably more complicated.

One complicating factor is that, not too surprisingly, different mouse models yield different results. Using Scott Zeitlin's knock-in mouse, Levine found only a slight, statistically insignificant tendency towards depolarization. And using the Aronin-DeFiglia mouse, he observed great variability among cells in these measures. Since disease progression varies between mouse models, Chesselet suggested that each model might have been at a different stage in the disease process, and thus yielded variable results.

These puzzling results, however, may soon be sorted out, since several participants showed interest in following up on Levine's studies. Hitoshi Kita proposed doing unit recordings in the striatum after

cortical stimulation and Edward Stern proposed doing intracellular, in vivo recordings to further investigate up and down membrane states, input resistance and the membrane potential of striatal cells.

### **The medium spiny cell: What makes it special?**

#### **Challenges and unresolved issues**

One of the key mysteries of HD is the large variability in the damage suffered by different cell types. In particular, a subset of neurons in the striatum seems to be the main target of the disease. What makes these cells so vulnerable? Is HD a global disease of neurons where some neurons are more damaged than others, or is it specific to striatal spiny cells?

At least two studies suggest that HD might be a global disease of neurons. Daniel Madison and Michael Hayden have recently found impairment of LTP in the hippocampi of two HD mice models. The mutant mice expressed less post-tetanic potentiation, decreased paired-pulse facilitation (Madison's findings), and released less glutamate during high frequency stimulation as compared to wildtype controls.

Elizabeth Abercrombie suggested that one possible explanation for these observations is that a common root cause affects all neurons in HD, but each neuronal type responds uniquely to the problem. Thus, striatal cells may simply be the most visibly affected. Most participants agreed that, regardless of the fate of other neurons, however, initial studies should focus on the spiny cells.

The heterogeneity of cell types in the striatum, however, presents a significant obstacle. The cells that are preferentially affected, the enkephalinergic neurons, are intermixed with other neurons in the striatum. Identifying and isolating this morphologically non-distinct subset is not easy. Several suggestions for overcoming the problem were presented.

#### **Potential solutions**

##### ***Identifying enkephalinergic spiny cells***

To identify the enkephalinergic neurons in the striatum, Levine has started back-labelling neurons with fluorescent beads. By injecting beads into the

substantia nigra (SN), he hopes to label substance P-containing neurons, and by injecting them into the GP, he hopes to label enkephalinergic neurons. Using infrared microscopy, he then plans to do electrophysiological studies on these identified neuronal populations. A potential pitfall of this approach, however, is the risk of causing damage during injection. Also, the populations are not completely pure: some neurons that project to the SN also project to the GP.

Tobin suggested moving away from whole tissues and using striatal cell lines. Surmeier, however, was skeptical of how well cell lines reflect the behavior of these cells in vivo. He mentioned that cells in culture often change their gene expression patterns which can lead to alterations in signaling cascades.

Instead, Surmeier proposed studying enkephalinergic neurons by patch-recording from single neurons in the striatum, and then performing RT-PCR on their contents. RT-PCR would serve both as a marker for identifying enkephalinergic neurons, as well as a tool to examine gene expression patterns. Since young animals are required for these experiments, Surmeier proposed using Bates mice, the model that expresses symptoms the earliest.

### ***Characterizing the pathology of enkephalinergic spiny cells***

To understand the pathology of HD, several participants stressed the importance of characterizing the morphological changes that enkephalinergic spiny cells undergo in HD. Using biocytin to fill neurons, Levine found that the spiny cells of Bates mice had smaller somas, fewer dendritic spines, and thinner dendrites than wildtype controls. Chesselet and Kita agreed that electron microscope studies were needed to further assess the cellular and subcellular changes caused by HD. Stern proposed removing a small piece of cortex and imaging striatal cells over the course of several days in anesthetized, stereotactically-restrained animals to monitor the movements of spines in vivo. Studies from Karel Svoboda's group have shown that synaptic plasticity includes the re-shaping of spines.

A surprising new finding that may offer clues to striatal cell susceptibility was presented by Shelbourne. Her group found that as their knock-in mice age, the CAG repeats in their striatal cells seem to become dramatically unstable. Their preliminary results show that repeats in the striatum are

particularly heterogenous and unstable as compared to other tissues. The finding is particularly intriguing since repeat instability is thought to be replication-dependent and neurons are non-replicating cells. Even glial cells, on average, turn over only once during a lifetime. Shelbourne noted that very little is known about the DNA repair mechanisms of neurons. One possibility is that the instability is related to transcription. Yet this does not explain why striatal cells are predominantly affected, since huntingtin is not particularly overexpressed in the striatum.

Tobin mentioned examples, such as retinitis pigmentosa, where deficiencies in DNA repair enzymes give rise to neurological sequelae. He also noted that HD fibroblasts are less able to repair damage from ionising radiation than normal fibroblasts. He suggested challenging the neurons with radiation to further investigate the instability.

But Chesselet pointed out that the defect may not necessarily map to a malfunction in repair mechanisms. Instead, striatal cells could be exposed to more DNA damage than other cells and, therefore, accumulate more errors with time.

Since it is generally accepted that the size of the CAG repeats is a primary determinant of the onset of pathology and symptoms, Shelbourne's observations may help explain why striatal cells are damaged so early in the disease process.

### ***Candidates to explain the unique physiology of enkephalinergic spiny cells***

Participants proposed several physiological parameters as possible culprits of enkephalinergic spiny cell vulnerability and experimental ways to test them. Surmeier suggested studying the expression of the inward-rectifier channels (Kir channels), since enkephalinergic neurons express a unique subset that slowly inactivate, and which may thus make them more susceptible to excitatory inputs. He was particularly interested in studying Kir 2.1 channels because an alteration in their activity could help explain Levine's findings.

In addition, Surmeier proposed using his patching/RT-PCR technique to investigate another unique feature of enkephalinergic neurons, D2 dopaminergic signaling. He speculated that D2 receptors might be constitutively active in HD. In some cases, HD patients appear to benefit from D2 blockers. And although still controversial, some D2

knockout mice become cataleptic, perhaps representing the opposite of the HD mice situation. This hypothesis might explain the elevated calcium observed by Michael Hayden's group in their YAC model of HD. Pinpointing a defect in this pathway would provide a well-defined molecular chain to work with, since it is well understood how D2 receptors are linked to L-type calcium channels.

Several participants stressed the need for further studies on the regulation of intracellular calcium. Beyond D2 receptors, Levine was interested in examining the possibility that NMDA receptors in HD mice allow increased calcium to flow into spiny cells. And to further characterize calcium behavior, Surmeier proposed studying the effects of hyperpolarization on calcium dynamics, and ion channel densities and their distributions. Stern suggested performing calcium imaging in the striatum and cortex of living animals. As with Surmeier, he favored using young (approx. 10 weeks old) Bates mice that are beginning to show formation of inclusions. Stern proposed using a calcium-sensitive dye to monitor both resting calcium and how it changes in response to electrical stimulation. Observing both cortical and striatal cells could provide insights into their relationship in disease progression. To achieve cell specificity, he described using these dyes as retrograde labels. Wilson mentioned that Jeffery Smith has used fura-dextran in this manner.

Graybiel and Wilson favored experiments looking at calcium buffering in spiny cells, a feature that may help explain their increased vulnerability. Graybiel's lab has recently cloned a set of calcium-binding proteins that are highly enriched in the striatum. She suggested comparing the expression of these proteins between normal and HD mice.

## The importance of circuits

### **Challenges and unresolved issues**

The spiny cells of the striatum form part of a complex circuit which includes various regions of the cortex, the GP, the SN, and the thalamus. And although the spiny cells are greatly damaged in HD, other cells, particularly cortical neurons, are also affected. Participants agreed that HD was probably best understood as a disease of neuronal circuits, rather than of isolated cells.

It has long been hypothesized that a defect in cortico-striatal projections could account for some of

the major symptoms of HD. In particular, it has been suggested that HD involves glutamate receptor-mediated cell death in the striatum. Local injections of NMDA receptor agonists, such as quinolinic acid, reproduce several of the neurochemical and neuropathological characteristics of HD. On the other hand, a recent study by Patrik Brundin's group showed that the Bates mice were actually more resistant to quinolinic acid excitotoxicity in the striatum than wildtype controls. One possibility is that the HD mutation causes a protracted, sublethal degree of excitotoxicity which primes the striatal cells to recruit increased defenses against excitotoxic death.

Levine's studies, however, suggest the opposite. Using a cell swelling assay, he found that the Bates mice's striatal neurons were more sensitive to NMDA agonists than wildtype controls. Some participants suggested that cortico-striatal connections might actually be disrupted or hypofunctional in HD. Levine found that he needed more current to elicit an evoked response from Bates mice's spiny cells than from normal controls when he stimulated the corpus callosum. This observation suggests that there are either fewer cortical afferents or that it is harder to induce them to fire. Abercrombie mentioned that administering the NMDA receptor blocker MK-801 to rats induced hyperactivity and uncoordinated locomotion reminiscent of HD. In addition, Chesselet said that by Golgi staining, cortical neurons of Bates mice appear very abnormal, often even more so than striatal neurons.

Dopamine inputs further complicate the picture. George Rebec described how his experiments in freely moving rats revealed that dopamine is a modulator of striatal function, regulating baseline activity as well as responses to glutamate. Surmeier noted that D2 receptors seem to shorten striatal cells' responses to glutamate. Thus, a defect in the dopaminergic system could affect glutamatergic responses. Chesselet pointed out that a very early abnormality in HD mice is the loss of D1 and D2 receptors.

Finally, GABA and acetylcholine may also play roles in HD pathology. And although cholinergic neurons in the striatum seem to be spared, Chesselet noted that the levels of choline acetyltransferase appear to be decreased in the striatal matrix of HD patients.

## **Potential solutions**

### ***The links between cells: Neurotransmitters and growth factors***

Although affecting different synapses in unique ways, a general impairment of neurotransmission might contribute to the deficiencies seen in the neurotransmitter pathways of HD mice and patients. Huntingtin has been found associated with synaptic vesicles and clathrin. It is possible that its mutated form interferes with the docking or release of synaptic vesicles. Chesselet has observed that although enkephalin staining of spiny medium cells appears normal, there is a substantial decrease in its mRNA levels in HD mice. Although the observations are not quantitative, they suggest that enkephalin release may be blocked. Also, Madison's studies in the hippocampus suggest that sustained release of neurotransmitter may be altered in HD mice. James Tepper suggested to Abercrombie performing dynamic dialysis measurements of neurotransmitters, such as dopamine and acetylcholine, in HD mice. Most neurochemical data available today come from tissue punches of HD patients, and are thus devoid of temporal information. Abercrombie agreed, but thought there was not compelling evidence to postulate either dopamine or acetylcholine as key players in HD.

Other possible sources of dysfunction in HD are the production and release of growth factors. Messenger RNAs coding for various growth factors have been found in striatal interneurons. A lack of one or several of these factors could explain the cell loss observed in HD. And since cortico-striatal connections appear impaired in HD, a decrease in cortical growth factors could also contribute to the loss. If, as suggested in the section below, striatal cells in HD mice behave in some ways like immature neurons, they may require more growth factors or be more susceptible to a lack of them. Surmeier proposed infusing growth factors in vivo to test this idea.

### ***The pathology of cortico-striatal connections: New insights and potential new therapies***

Comparing HD striatal function with normal striatal function during development, prior to the establishment of mature cortico-striatal connections, proved very informative. Early during development (rats less than about 4 weeks of age), medium spiny cells share several of the characteristics described by

Levine in HD mice: they have fewer dendritic spines and lack inward rectifying currents. In addition, as pointed out by Chesselet, during normal development, animals experience a physiological chorea which may coincide with these attributes. Thus, mutant huntingtin may either affect normal development or produce a reversal in the adult animal. As noted by Eric Nisenbaum, experiments examining these features in mouse models with different CAG repeat lengths may provide a pathological correlate with severity of disease and/or age of onset. At the very least, he thinks these data, if confirmed, suggest an anatomical measure (i.e., spine density) which could be examined in postmortem tissue.

To reconcile the apparently contradictory results suggesting that cortico-striatal connections are both hyperfunctional and hypofunctional in HD, Tepper suggested that not all cortico-striatal pathways may be affected by HD in the same way. The pathway that innervates the spinal cord and sends off ipsilateral collaterals to the striatum, for example, might be hyperfunctional, while the crossed pathway that does not project beyond the striatum may be hypofunctional. Retrograde tracing from the spinal cords of HD mice would be one way to test this hypothesis.

To explore the role of glutamatergic function in HD, Nisenbaum proposed examining glutamate receptors in HD mice. He suggested using ligands for specific glutamate receptor subtypes in combination with RT-PCR to study glutamate receptor subunit expression in brain slices and isolated neurons. Referring to the similarities of HD with development, Nisenbaum speculated that changes in glutamate receptors like those seen in development, might play a role in HD. For example, the amount of calcium that can pass through GluR2 receptors changes during the course of development. It is possible that the increased calcium seen in HD striatal neurons is the result of a similar alteration in glutamate receptor function.

If indeed a lack of glutamatergic input from the cortex causes the abnormal function of the striatum in HD, boosting glutamatergic transmission might be therapeutic. Abercrombie suggested testing an antagonist to glutamatergic presynaptic autoreceptors to potentiate glutamate release. In this manner, increased glutamate release could be restricted to active synapses.

Another innovative therapeutic proposal was proposed by George Rebec. He suggested modulating glutamate activity with ascorbate (vitamin C). Ascorbate is found in particularly high concentrations in the extracellular fluid of the striatum. Increasing evidence suggests ascorbate can protect against glutamate-induced neurotoxicity and may also regulate glutamate-induced activation of striatal neurons. In addition, fluctuations in striatal ascorbate are closely related to behavioral activation. Rebec suggested examining ascorbate levels in the striatum of HD mice and correlating the levels with behavior. He also suggested performing parallel studies in HD patients. To test ascorbate's therapeutic potential, he proposed manipulating ascorbate levels in HD mice and assessing the effects on behavior. The known neuroprotective effects of antioxidants and ascorbate's availability as a dietary supplement add to ascorbate's appeal as a potential therapeutic compound.

In addition to the proposals aimed at directly studying or regulating glutamatergic inputs to the striatum, proposals to study the role of modulators of glutamatergic responses were also put forth. Tepper hypothesized that the abnormal response of striatal cells to cortical stimulation might be the result of an impairment in GABAergic regulation of spiny cells by interneurons. When striatal cells of HD animals respond to cortical stimulation, they often show exaggerated responses. The depolarized state and increased input resistance of striatal cells could be explained by such an impairment. To test the hypothesis, Tepper suggested doing whole-cell recordings in HD brain slices to monitor spontaneous IPSCs and their regulation by acetylcholine, which is known to increase the frequency of interneuron firing. He also proposed carrying out paired recordings from interneurons and spiny cells to assess whether their interaction is abnormal in HD mice, and complementing these experiments with pharmacology to further probe the postsynaptic responses of spiny cells. If there is a GABA defect, anti-epileptics or other GABA mimetics could provide a means to control symptoms.

An often overlooked, and potentially crucial, source of cortical input to the striatum was brought to the attention of the group through an observation made by Lucy Brown of the woman who participated in the workshop. Brown noticed that her choreic movements seemed less pronounced on the left side of her body, which was being stroked by Wexler. Since the basal ganglia receive sensory inputs in

addition to motor inputs, Brown speculated that the sensory input from stroking might somehow help reduce uncontrolled movements. If confirmed, a transcutaneous electrical nerve stimulator (TENS) might help patients in the early stages of the disease. TENS stimulators are used for the treatment of chronic pain and can be worn all day and programmed with a variety of different pulses. Graybiel mentioned that in other movement disorders, such as Tourette's syndrome, stroking sometimes helps patients break out of obsessive trains of behavior.

In addition to their therapeutic potential, somatosensory inputs offer simple probes into basal ganglia function. Although HD patients don't show major sensory deficits, their responses to vibrational stimuli on their fingertips are slightly altered as assessed by PET imaging, and they are less sensitive to pain. Brown suggested using an anterograde tracer in conjunction with 2-deoxyglucose (2-DG) as a metabolic marker to study the effects of sensory and electrical stimulation on different brain regions in HD mice. More generally, she plans to use 2-DG to carry out a survey of the whole brain, with particular emphasis on the striatum, GP, and the cortex. By comparing several mouse models, she hopes to find shared deficiencies that might guide other anatomical and electrophysiological experiments. Ideally, parallel PET studies in humans would allow valuable comparisons between the mouse models and HD patients. Tobin suggested using a dopamine D1 receptor ligand for PET which can provide image resolution in the millimeter range. If necessary, micro-PET could be done on mice to complement the 2-DG studies.

### ***Beyond cortical inputs: Basal ganglia circuits as a whole***

If HD is a disease of circuits, then understanding how basal ganglia circuits operate under normal conditions is key. A theoretical model presented by Hagai Bergman offered a novel framework for understanding how the basal ganglia work.

On first inspection, it is not obvious why the basal ganglia even exist. Basal ganglia receive their input from the cortex and send their output to the cortex, a structure which uses more than 99% of its connections for internal communication. The traditional view, the action-selection model, proposes that the basal ganglia's job is to select single actions for execution from the many possible actions generated

by the cortex. This model is based on anatomical observations that suggest strong lateral connectivity -- it assumes striatal neurons mutually inhibit each other. Yet recent physiological experiments have failed to show correlated activity or lateral interactions in either the striatum or the GP.

Bergman thus developed a model which is consistent with the new physiological findings. The model uses neural networks and incorporates key aspects of basal ganglia connectivity. In this model, the basal ganglia don't select data, but rather compress it (or dimensionally reduce it). Among other things, this helps explain the 100-fold reduction in the number of neurons from the cortex to the striatum, and the additional 100-fold reduction from the striatum to the GP.

The compression occurs in a reinforcement-dependent manner which mirrors experimental findings showing that the basal ganglia process reward-related signals. The relative significance of an input is determined both by its novelty and by its probability of predicting reward to the animal. Thus, correlated firing is expected to occur only when a new association is being learned. In an unchanging environment, as used in most experiments, firing should be uncorrelated. Bergman has tested and confirmed these predictions using multi-electrode recordings in awake, behaving monkeys.

When this fine-tuned, data compression circuit is disrupted by disease, Bergman's model predicts specific ways in which the system will fail. For example, in Parkinson's disease, the striatum is receiving low levels of dopamine which would correspond to a state of continuous disappointment, where predicted rewards fail to occur. Lacking the reinforcement signal, the neurons are unable to decide which associations are important, and so they continually reorganize according to the latest incoming information. Administration of L-dopa helps restore basal levels of dopamine, but since it isn't properly matched with external events, it introduces noise into the system.

Several participants agreed that Bergman's model also seemed to fit well with what is known about HD. Wexler mentioned that HD patients seem to have a massive disruption in their capacity for selecting actions -- they have extra thoughts, movements, and a striking tendency to get distracted.

Chesselet proposed testing the role of the GP in Bergman's model. As shown by Tepper, one of the most potent regulators of dopamine firing in the SN is the GABAergic input from the GP. Because the GP is regulated by the striatum, one of the effects of abnormal activity in the cortex and striatum could be the dysregulation of the dopaminergic pathway. This dysregulation could, in turn, lead to improper associations resulting in abnormal movements.

Tepper said he could probably test this idea pharmacologically by applying GABA receptor antagonists. He also suggested doing whole-animal extracellular recordings in the GP and SN of Bates mice to characterize their firing patterns. In addition to providing relevant data for Bergman's hypothesis, these experiments could provide more general data on the alterations in circuit behavior caused by HD. Kita strongly agreed with this approach, stating that obtaining baseline information on basal ganglia circuits was, in his opinion, a top priority. One challenge Tepper will face, however, is setting up these experiments in mice, especially developing mice which are small and have very soft skulls.

To further test his model, Bergman plans to stimulate the SN of monkeys both focally and transiently. If his model is correct, he expects to induce dyskinesia with random stimulation. He is also interested in finding out whether this stimulation induces cognitive deficits and whether, over time, the cortex undergoes any compensatory changes to adjust to this pathological situation. To test the circuit in more detail, he proposed to activate neurons in different locations.

One potentially promising prediction from the model is that because the system functions as a network, it should be able to compensate for focal disruptions. In fact, after small lesions, the basal ganglia generally recover within a month. Thus, treatment of HD may be possible even after initial symptoms have begun to surface. And given that at least some of the pathological changes expressed by HD mice appear to be reversible, the odds of effective intervention after the onset of symptoms may be even better. According to Wexler, four months after the mutant huntingtin gene was turned off in the inducible Tet-OFF model, aggregates in the striatum seemed almost to disappear.

But reversibility is not very useful without a therapy. A potential therapy proposed by Bergman was implanting a deep brain stimulator into the GP or

subthalamic nucleus. By varying the frequency and voltage of stimulation, conditions could be optimized for individual patients. Bergman speculated that besides offering direct relief of symptoms, stimulation might change the progression of the disease by retarding downstream plastic changes in the cortex. In addition, because the stimulator can be turned off easily, it represents a low risk intervention. Chesselet cautioned, however, that the insertion of the stimulator could cause tissue damage. Still, Bergman ranked his proposal as a top choice compared to fetal transplants or CNTF-producing capsules. He speculates that patients with advanced HD, who have no time to spare, would be more than willing to take the risk.

The dynamic and adaptive nature of the basal ganglia model presented by Bergman was also consistent with Graybiel's recent observations in freely-moving rats. Graybiel studied the firing patterns of striatal neurons in rats learning a T-maze. As the rats learned, their firing patterns changed. At first, many neurons fired as the animal prepared to turn in response to a tone. At later stages, firing was maximal as the rat started the maze. Still later, responses were increased when the rat reached its goal. At the very end of training, most firing was concentrated at the beginning and end of the maze run. The firing patterns described applied to the population as a whole, indicating that the striatum is truly a highly dynamic structure.

Graybiel speculated that this reorganization may underlie habit formation. By regulating habitual behaviors, the basal ganglia free the cortex to deal with higher cognitive behaviors. Wexler agreed with this view adding that HD patients have trouble combining stereotyped tasks with higher cognitive tasks. HD patients often find it difficult to hold their hands out and simultaneously count backwards, for example, and are easily distracted. In addition, Wexler mentioned that imaging studies suggest that HD patients rely on their cortex for performing habitual tasks, which in normal humans are controlled by the basal ganglia.

### ***In vivo electrophysiology and neurochemistry in freely behaving animals: A must***

Participants overwhelmingly agreed that electrophysiological and neurochemical studies on awake, behaving animals were sorely needed. As stated by Rebec, these studies offer three important advantages: 1) the intactness of neuronal circuits, 2) a

lack of complications associated with general anesthesia, and 3) the possibility of directly correlating behavior with physiology.

Rebec proposed combining single-unit recordings with iontophoresis and various pharmacological manipulations in normal and HD mice. He suggested, for example, recording how neurons in the GP and the SN pars reticulata respond to somatosensory stimulation, open-field locomotion, and discrete head and limb movements. Once baseline responses were defined, he proposed using iontophoresis to stimulate the neurons with GABA, glutamate, and dopamine. A specific experiment of this type proposed by Wilson was to record in the SN pars reticulata, the output structure of the basal ganglia, during a head movement task. Understanding how firing patterns are altered in HD mice might reveal important aspects about how the circuit malfunctions.

The ultimate experiment in this category, however, is to perform multiple recordings on freely behaving animals. Studies of movement and visual receptive fields have illustrated that through analyzing the behaviors of neuronal ensembles, one can obtain a much more accurate picture of neuronal encoding than from the study of single neurons. Graybiel has ample experience with multiple recordings in behaving rats, and is now setting up similar recordings in mice. So far, she has successfully recorded from one mouse. If these experiments can be set up reproducibly, they promise to shed a bright light on basal ganglia circuitry and how it's disrupted in HD.

### **The chicken-and-egg problem: What is the temporal sequence of events in HD?**

#### **Challenges and unresolved issues**

Perhaps the most gnawing issue in HD research today is distinguishing primary from secondary effects. As illustrated in this report, a growing list of HD-associated abnormalities is being compiled, yet very little is known about how these abnormalities are linked to each other through cause and effect. It is still unclear, for example, how the three main pathologies of HD -- cell loss, inclusions, and altered behavior -- relate to each other.

Although the mouse models should greatly assist in clarifying this situation, the answers will probably be complex. Each model shows different combinations of pathologies, and meaningful correlations have been

difficult to extract. The late-onset of the disease further complicates the problem. Progressive build-up of small alterations can be hard to detect, especially at early stages.

How the number of repeats helps determine age of onset is also unclear. One clue was provided by Chesselet who noted that there is more widespread pathology in juvenile HD than in adult HD -- several brain regions in addition to the striatum and cortex, such as the cerebellum, are affected in the juvenile form. If different cell types have different vulnerability thresholds, it is possible that the increased repeats in juvenile HD result in levels of toxicity that exceed more cells' thresholds. But why more repeats are more toxic is still unknown. And why the juvenile form shows symptoms of rigidity, whereas the adult form shows chorea is also a mystery.

### **Potential solutions**

The most direct way to begin to establish the sequence of events that leads to HD symptoms and pathology is to follow the course of the disease, starting at as early an age as possible. The mouse model that most directly promises to enable these studies is the inducible Tet-OFF model. By controlling when the mutated huntingtin is expressed, researchers should be able to follow the course of the disease precisely. Tepper proposed a developmental study of inducible mice using intracellular recordings and looking at the morphology of the striatal spiny cells. A key question that might be answered by these studies is whether mutated huntingtin starts having its deleterious effects during development are such that the maturation of the brain is affected, or whether it acts only on the adult, fully formed system. In either case, pinpointing the first signs of abnormality will provide key clues towards uncovering the primary events that unleash the many aspects of HD pathology. Several researchers agreed that, provided they had sufficient mice, they would do time series for their particular experiments.

If mutated huntingtin starts causing trouble after development is complete, it might be possible to prevent disease progression by eliminating huntingtin in the mature animal. Nisenbaum proposed using ribozymes as a potential therapy to remove huntingtin in adult animals. Since huntingtin is known to be required for development, this procedure could only be carried out after development was complete.

Another tool that may help elucidate the temporal progression of HD is the use of viral vectors to express mutant huntingtin in specific brain regions. This technology, which allows the generation of rat and monkey models of HD, may also help understand disease progression, as suggested by Chesselet. For example, one might get an indication that the cortex is the source of primary pathology, if incorporating the mutant gene into the cortex induced full-blown striatal pathology.

Searching for more subtle pathologies may also help reveal primary sources of disease. Electron microscopic characterization of cortical, striatal, SN and GP neurons was suggested by Chesselet to Kita as a means of further understanding the spatial and temporal pathology of HD. EM may reveal early abnormalities that have been missed at the light microscope level.

In a similar manner, Nisenbaum suggested using DNA microarrays to screen gene expression in HD basal ganglia. This broad-base approach might reveal early and subtle alterations in cell function. Ethan Signer noted that these studies were already underway and that initial results revealed changes in the expression of signal transduction proteins and neurotransmitter receptors.

Several participants called for biochemical and cell biological data. Many predicted that gaining an understanding of both the normal and mutant function of HD, would greatly accelerate the resolution of many of the mysteries of HD. Tepper commented that if there is any indication from the Tet-OFF mice that pathology and symptoms can be reversed through the elimination of aggregates, then the highest priority should be to attack the aggregates with biochemical or molecular techniques. Tobin agreed but explained that, although cell and molecular biologists have made great advances, they are still unable to explain most of the fundamental aspects of HD. Therefore, he predicted that the best path towards understanding HD was to enlist the concerted efforts of cell biologists, molecular biologists, anatomists, and physiologists.

### **Possible partnering with industry**

To accelerate the development of a cure, participants discussed the possibility of partnering with industry. Signer provided an informative summary of the factors involved.

From the standpoint of a pharmaceutical company, the main question regarding HD is: how much money will it make? A rough calculation of HD's earning potential yields \$200 million a year. Taking all CAG repeat disorders together might increase the estimate as much as two-fold. Still these amounts are well below the standard minimum – one billion dollars – that large drug companies require for investing in a drug. Nisenbaum thus suggested approaching smaller biotech companies.

But according to Signer, a drug for HD is attractive as an investment for reasons beyond these simple calculations. First, unlike most diseases, the diagnosis for HD is almost 100% accurate. This means that practically all cases can be identified early and treated immediately, starting from birth (if not even in utero). Another consideration is that, in reality, most drugs do not provide their companies with one billion dollar earnings. As Nisenbaum said, "Not everything is Prozac." Finally, the further along a drug is on the path to discovery, the greater the likelihood that it will be successful. Powerful *in vitro* and *in vivo* systems, including biochemical assays and mouse models, already exist for the testing of candidate drugs for HD.

Participants suggested several alternatives to marketing HD. Tobin raised the possibility of presenting HD as a member of a large group of diseases -- including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis -- which are all characterized by the formation of protein aggregates in the brain. But several participants thought this might be too broad of a category. Instead, Signer proposed categorizing CAG diseases by the involvement of caspases or based on whether the initiating events of the disease were triggered by similar macromolecular interactions.

Another possibility raised by Nisenbaum was the development of versatile drugs that may target a wide variety of diseases, such as modulators of glutamate. An inverse approach, proposed by Signer, was to search the medical records for cases in which HD patients, taking medications for other conditions, noticed improvements in their HD symptoms. Perhaps existing, FDA-approved drugs are useful for the treatment of HD.

## Conclusions

The conceptual and technical insights that emerged from the HDF workshop are poised to accelerate the development of a cure for HD. Although a consensus was not reached regarding what "HD should look like in a mouse," participants agreed that studying the basic physiology and pharmacology of the existing mouse models should ultimately yield answers to this question. New and more subtle ways of studying behavior should also provide improved criteria for evaluating and comparing the models. And for those experiments that require rats or monkeys, viral vectors may offer a practical way of generating alternative HD models.

Participants also suggested several novel ideas on how to probe the uniqueness of the medium spiny cell. Single cell recordings coupled with RT-PCR, for example, offer a way of identifying cells by their expression of enkephalin, while studying their electrophysiology and patterns of gene expression. A new hypothesis to help explain the early damage of striatal cells was provided by preliminary results showing a striking instability of CAG repeats in striatal cells. Other promising candidates, including inward-rectifying potassium channels, dopamine D2 receptors and calcium-binding proteins, were also nominated.

The importance of analyzing basal ganglia circuits surfaced repeatedly. A neural network model of basal ganglia function and multi-electrode recordings of maze-running rats provided new insights on normal basal ganglia function and testable predictions of how disruptions might cause HD symptoms. Based on a discussion of these data, the implantation of a deep brain stimulator in the GP or subthalamic nucleus was proposed as a potential therapy. Furthermore, data from electrophysiological and morphological experiments suggested that striatal cells in HD are, in several ways, similar to immature neurons. This insight spurred the proposal of several new hypotheses and experiments. Rarely considered factors, such as somatosensory input to the basal ganglia and the regulation of glutamate receptor responses by vitamin C, enriched the discussion of both pathology and potential therapies.

Establishing the elusive chain of events that triggers HD progression emerged as a challenging, but approachable task. Developmental studies of the electrophysiology, pharmacology, and morphology of basal ganglia and cortical cells should ultimately shed light on the origins of pathology. In particular, the

inducible Tet-OFF mouse model promises to accelerate and extend these findings. It should also provide a way of testing the reversibility of various pathologies.

These insights illustrate how well the workshop succeeded at bringing together new perspectives and suggesting new experimental directions. The participants varied backgrounds enriched the

discussions and led to novel ideas unlikely to surface in more specialized meetings. More importantly, the workshop moved the search for a cure of HD one significant step forward.

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