CHDI Basal Ganglia Working Group Meeting
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Session 1: *In vivo* investigation of basal ganglia function in HD models

George Rebec of Indiana University presented his work looking at *in vivo* neuronal activity in awake, behaving mice. He characterized the problems in animals meant to model aspects of HD as "a failure to communicate." (Here he presented recordings from R6/2 mice but reports that the results have been consistent in other strains with mutations to the huntingtin gene.) The properties of individual neurons in striatum were different in the HD mice; although they still occasionally fired in bursts, this activity was reduced, and coincident bursting among groups of neurons was not seen in HD mice as it is in wildtype (WT). Rebec also recorded local field potentials (LFP) and saw that, specifically during quiet rest behavior, HD mice displayed a high-frequency oscillation at about 32 Hz—in the low gamma range—that was not seen in WT mice. In addition, the striato-cortical coherence of firing was disrupted in HD compared to WT mice.

In collaboration with William Yang, Rebec examined EMX-Cre1 mice, which have a reduction in full-length mutant huntingtin (mHtt) [derived from BAC-HD mice] specifically in cortical pyramidal neurons. Striatal neurons—and all other cells—still express the mutant protein. Bursts of activity are seen in WT mice, but the Cre mice neuronal activity pattern appeared to be intermediate between WT and the BAC-HD mice, which might suggest that the restoration of normal HTT in cortical neurons has partly rescued the normal firing pattern in the striatum. In summary, Rebec found communication problems within the striatum, within the cortex, and between the brain areas.

Anthony West of Rosalind Franklin University reported his preliminary findings about direct- and indirect-pathway signaling properties in the BAC-HD transgenic rat. These *in vivo* recordings in awake, behaving animals represent the first look at the electrophysiological properties of the full-length transgenic HD rat. West recorded from medium spiny neurons in the striatum after stimulating orthodromically in the cortex or antidromically in the substantia nigra (SN). West identified MSNs that responded to this nigral stimulation as direct-pathway neurons, which express D_1 receptors. (Ann Graybiel asks whether this stimulus affects both SNr and SNc parts of the substantia nigra; West did not specify in his presentation.) In tgHD rats, only about half the number of neurons responded to cortical stimulation compared to WT, suggesting a general decrease in MSN evoked activity.

The early data suggest that fast-spiking interneurons (FSI) may behave in the opposite way: they appear more responsive. With antidromic stimulation in the SN, direct-pathway neurons in HD rats required less current density to evoke a response, perhaps indicating higher excitability in the MSN terminals.

West also investigated the effects of a phosphodiesterase (PDE)10 inhibitor. Thirty minutes after a subcutaneous injection of the drug, the proportion of MSNs that responded to cortical stimulation increased significantly. In addition, a few previously silent, unidentified (with respect to direct/ indirect) neurons began a sustained spontaneous firing about 20 minutes after the drug came onboard. West will continue to collect data about both the native properties of the HD rat basal ganglia neurons as well as effects of PDE inhibitors.

Margaret Levin of Galenea, a biotechnology company, described their overall approach to developing novel CNS disease therapeutics. Using technology Galenea developed, they can measure *in vitro* synaptic transmission in cortical cultures from Q175 and BAC-HD mice, and use those assays in a high-throughput screening platform for drug interventions; they call this platform the MANTRA system. They hope to identify synaptic signatures associated with the disease model that—ultimately—might be affected by a compound.

In another platform, they hope to identify and perhaps affect signatures in brain slices recorded with a multi-array of electrodes, perhaps including plasticity or oscillation assays. The next step includes the work she presented: network activity *in vivo* from awake behaving mice, including three models of HD. What Levin initially called EEG recordings are local field potentials (LFP) in the mice in an area referred to as the prefrontal cortex (PFC), which Levin described as frontal association cortex. Finally, human electroencephalographic (EEG) signatures might be identified in HD patients, which could correspond to changes in cognitive activity. Kevin Spencer at Harvard works with Galenea in making the human recordings. Ideally, Galenea hopes to be able to collect enough data in these various levels—single cells, slices, local field potentials from animals, and EEG data from humans—to be able to form links between them. Though CHDI's goals in working with Galenea do not include linking the platforms, so to speak, they hope to gather informative data about HD mouse models and possibly patients within individual platforms.

Levin presented some data about LFP recordings in the Q175 heterozygous and homozygous knock-in mice at four and six months of age and the BAC-HD transgenic mice at three and five months. The data were preliminary and somewhat perplexing. They used three behavioral paradigms: ongoing recording during habituation to an open-field; an auditory steady-state response (ASSR) condition; and an object-recognition task. During the open field recordings, Levin found significant differences in LFP activity in the theta-band range. These data were collected over 15 minutes of exploratory behavior, and the results from several sessions over three days were compiled. They also recorded mouse behavior at the same time, but did not consider those results in the analysis of the data. Levin saw significant differences in the level of activity in the theta range—around 30 Hz—between the WT and mutant mice. The effects were in opposite directions in the heterozygous and homozygous Q175 mice, and the changes seemed to reverse over time. Levin said that she would welcome input about how to analyze the data. With respect to the different findings in the hetero- and homozygotes, Beaumont pointed out that the homozygous mice might display completely different electrophysiological properties because the mutation may have affected development. Therefore, they might never display an electrophysiological signature like a wildtype mouse.

Ann Graybiel and others strongly recommended that Levin account for the specific behaviors displayed by the mice and to include that as a factor in the data analysis. The results seen in the theta range of activity recorded in the frontal cortex likely contributes not only to movement behaviors but also anticipation states without movement. In addition, Graybiel reports that she and others (e.g. Matt Wilson) have shown that cortical activity can be coherent with both the hippocampus and the striatum, but the coordinated activity depends highly on behavior. "They're coherent depending on what the animal is doing. It depends on when they're moving or not," said Graybiel. It also depends on the animal's current learning and knowledge stage, she added. Rebec's data also hinted at the importance of behavioral state. As mentioned above, the Galenea study included data about the mice's moment-to-moment behaviors, and these should

be included in future analysis. An additional, possibly confounding factor is that WT mice appear to be more physically active than the HD mice, and in some analysis the data have been normalized to that movement.

Lynn Raymond expressed concerns with the way the data had been selected and presented; simply homing in on theta activity that increases and decreases with phenotype progression was not very informative. As more data can be collected over a full time course in multiple models, as Rebec has done, hopefully patterns will then emerge, Raymond said. Levin agreed and recognizes that theta activity is simply where they've seen changes with this initial pass. Raymond also raised concerns about the strategy of recording from PFC in the mice, and wondered if it would be expected to even show alterations in the mouse models. (See Discussion Points for more on this topic.) Levin said one reason for choosing cortex is that human EEG data reflects cortical activity and can be coordinated with cognitive readouts. Rebec added that he has done some recording in the HD mouse PFC and saw similar changes to what he's seen in motor cortex in terms of individual neuron properties. Again, mHtt-related patterns emerged when neural activity was analyzed according to the animals' specific behaviors, which also differ in WT and HD mice.

Baljit Khakh suggested that Levin first establish a baseline of mHtt-related neural activity changes, especially if you hope to link the multiple assays to one another or to human disease. First, take a well-established mouse like the R6/2 at a stage when the phenotype is strong, and compare that to a wildtype mouse, he recommended. "Do the same two experiments in both mice and find signatures. Wouldn't that give you confidence in these assays?" To this, Graybiel added, "the activity of striatal (and cortical) neurons are highly task-dependent." So for Khakh's suggested inquiry, it would be important to define the goal. Such experiments throughout development might aid in describing the onset of a defect, but other methods might be required to characterize a late-stage pattern.

Session 2: Basal ganglia function in HD models

Donald Faber of the Albert Einstein College of Medicine described disease model-related changes in the intrinsic properties of individual MSNs in the R6/2 mouse. Faber descried a homeostatic mechanism that limits neuronal excitation; he called it fast Activity-Dependent Homeostasis (fADH) of intrinsic excitability. In wildtype MSNs, a repeated pattern of stimulation and rest resulted in a spike train that adapted with reduced firing rate and increased variability. The time constant of the adaptation is around 10 ms, and it requires only minimal stimulation to achieve the effect. In the R6/2 neurons, however, neither the adaptation nor the variability increase occurred. As a result, neuronal firing became "clock-like," said Faber. He has not yet distinguished between direct- and indirect-pathway MSNs, but hasn't seen an indication that they will look different. He then linked the calcium-independent process to the "M current" which passes through KCNQ potassium channels. The loss of this voltage-dependent current with disease could contribute to MSN hyperactivity, which could potentially contribute to neurodegenerative processes. The KCNQ channel-activating drug Retigibine reduced phenotypic behaviors in R6/2 mice, though the behaviors were not specifically described.

Faber described a different picture from that of the "failure to communicate" scenario suggested by Rebec's data (although it could square with data presented by Surmeier, see below). Strikingly, Faber found that synaptic connectivity was increased in the R/62 mice compared to wildtype, and dye-coupled connections between striatal neurons were nearly tripled in R6/2 compared to WT. There was some discussion about the electrophysiological and biochemical events that might underlie the loss of adaptation and how to best target the process with a potential therapy.

James Surmeier of Northwestern University focused his research at the level of synaptic connections of MSNs. With the loss of cortically supplied BDNF, striatal neurons are thought to undergo synaptic plasticity changes that contribute to neurodegeneration. Surmeier developed a method to examine synaptic potentiation in postsynaptic striatal neurons without involving the presynaptic cortical neurons. He used caged glutamate released by optical stimulation to stimulate striatal neurons. He characterized a form of LTP that was found in about 30-40% of exclusively cortical-striatal terminals in WT animals.

The potentiation appeared similar in both direct- and indirect-pathway neurons. In the BAC-HD and Q175 mice, however, the LTP was lost selectively from indirect-pathway neurons. By two months, the proportion of cells displaying the LTP in BAC-HD mice had fallen to 10%; by six to eight months, it had fallen basically to zero. Similar results were seen in the Q175 heterozygous mice. If LTP were impaired, one would expect the size of synaptic responses at cortico-striatal synapses to be diminished. Surmeier saw the amplitude of evoked minis did indeed drop in the HD mice, consistent with the plasticity data and with the observed diminished responsiveness of indirect neurons to cortical input.

Can the plasticity be rescued? Surmeier reported that manipulation of the pathway through the TrkB receptor or through receptors for fibroblast growth factor (FGF) led to restoration of the plasticity. He then sorted cells according to D1- or D2-receptor-driven GFP expression, and thereby isolated pure populations of direct- or indirect-pathway cells. Quantitative RT-PCR revealed no change in TrkB mRNA, but FGFR and PTEN mRNAs were increased. He hypothesizes that molecules including p75 and PTEN are somehow conferring a standing inhibition over this pathway that lead to synaptic potentiation, and when that inhibition is disrupted, the LTP is restored.

Mark Bevan of Northwestern University presented complementary data to Surmeier's, from investigations in the "extra-striatal basal ganglia," particularly the external globus pallidus (GPe). Bevan described the circuitry of the basal ganglia, including the subthalamic nucleus, which receives direct input from the cortex. His investigations downstream of the striatum can lend clues as to whether the reciprocal arrangement of the circuit "powerfully influences basal ganglia output" and thereby motor behavior, he said. His initial aim is to identify electrophysiological properties of these neurons in putative HD model animals with an eye to maladaptive changes that could potentially underlie the motor phenotype. (This issue is somewhat complicated by the differences in motor behaviors between the mutant mice and humans with HD; see Discussion Points.) Bevan noted that he is normally guided (in Parkinson's disease research) by *in vivo* findings, but in HD, only one report (Starr et al. 2008) exists, which found activity "consistent with the classical idea of how GP and STN relate to the hyperkinesia of HD."

Neurons of the GP fire autonomously, but their pattern is modulated by synaptic inputs. Bevan has characterized the ion channels that contribute to their firing, including NaV, HCN, KV, CaV, and SK channels. In *ex vivo* slices using cell-attached patch recordings, they saw that spontaneous firing was significantly elevated in the BAC-HD and Q175 mice. Bevan suspected that it was calcium-dependent and likely worked through the CaV2 or SK channels; both were downregulated according to qPCR in the HD neurons. The increased autonomous activity indicates a "pro-hyperkinetic" phenotype. The excitability increase was independent of action-potential signaling by upstream neurons. Bevan recorded GABAergic mini's, which indicated no change in connectivity or presynaptic release probability. In similar recordings in the subthalamic nucleus (STN), Bevan also saw a reduced rhythmicity of autonomous firing. Recordings in STN are sensitive to dialysis, meaning that they degrade in some way during standard electrophysiological recordings, which allow the cell's contents to diffuse into the pipette, often disrupting intracellular signaling pathways. Therefore, cell-attached perforated recordings will be required, which keep the cell's molecular signaling intact. Bevan described the downstream result: the neurons are less able to resist the hyperpolarization by inhibitory synaptic inputs, which appears to occur through downregulation of the HCN channel, making the STN more sensitive to inhibition by the GP.

Though these *ex vivo* slices didn't allow for the consideration of cortical inputs (which directly synapse in the STN), other work has shown (Shen & Johnson 2010) that activation of K_{ATP} channels provides a negative feedback loop that might drive striatal neuropathology. Could it drive changes within the STN as well? The changes might have multiple contributions, including increased synaptic glutamate, different expression of NMDA-type receptor subunits, or disrupted astrocytic homeostatic management of glutamate through transporters. Questions about the cortical contributions might be addressed with optogenetic methods. Finally, nitric-oxide signaling may also contribute to the electrophysiological alterations. Together, the results indicate that mutant huntingtin has cell-autonomous effects on basal ganglia neurons outside the striatum and cortex.

Sheng Zhong of PsychoGenics characterized the firing properties of neurons from the GP and STN, from which he made simultaneous, single-unit recordings in anesthetized wildtype and BAC-HD transgenic rats. He detected three previously described patterns: regular, irregular, and burst, in both structures. The dual recordings also revealed the dynamic interactions between GP and STN neurons, which sometimes fire in phase or out of phase with one another, and which also display adaptive changes with relation to one another. In the GP, the firing rate of neurons in BAC-HD rats compared to WT was slightly but not significantly increased. The pattern also shifted from bursting to more regular firing. In the STN, in contrast, BAC-HD rats displayed a lower firing rate and the pattern shifted to more bursting activity.

In a pilot study, Zhong applied the CHDI PDE-inhibiting compound used by others. In male WT rats, the compound caused a three-to-four-fold increase in firing rate in STN (but not GP) neurons. In female rats, however, no effect was seen on firing rate, showing a striking and surprising sex effect. An initial experiment on the BAC-HD rat suggests that the compound alters firing properties in STN neurons. Zhong plans to continue to investigate and expand on these results and to determine the expression pattern of PDE10 in the GP and STN. It remains unclear whether the compound affects neurons outside the striatum, or whether any extra-striatal drug effects would improve mutation-related basal ganglia abnormalities.

Session 3: Consequences of impaired striatal innervation in HD models

Anton Reiner from the University of Tennessee Health Science Center, Memphis discussed his characterization of basal ganglia circuitry in wildtype and heterozygous Q140 mutant mice. He first described the two types of cortical neurons that provide input to the striatum. The intra-telencephalic (IT) type corticostriatal neurons have their perikarya in the upper part of layer 5, and they preferentially innervate the D1-expressing direct pathway neurons of the striatum that are thought to play a role in initiating a planned movement. In the lower part of layer 5 are the pyramidal-tract (PT) type corticostriatal neurons. The PT-type corticostriatal neurons are also known as the upper motor neurons of cerebral cortex that project to spinal cord, and they give off collaterals in the striatum that preferentially end on the D2-expressing indirect pathway striatal neurons that are thought to inhibit movements in conflict with planned movement. By using best fit algorithms to combine the size frequency distributions of IT-type axospinous terminals, PT-type axospinous terminals, and thalamostriatal terminals, Reiner was able to estimate that 65% of input to D1-expressing striatal neurons in rats is from IT-type cortical neurons and about 35% is from thalamus. D2-expressing neurons were estimated to receive about 55% of their axospinous input from PT-type cortical neurons, 25% from IT-type, and 25% from thalamic neurons.

Reiner then used electron microscopy (EM) and antibody labeling of vesicular glutamate transporters, dopamine receptors, and choline acetyl-transferase (ChAT) to characterize the changes in corticostriatal and thalamostriatal synapses over time in WT and Q140 heterozygous knock-in mice. Note that presynaptic terminals from the cortex contain the vesicular glutamate transporter vGluT1 , whereas presynaptic terminals from thalamus contain the vesicular glutamate transporter vGluT2. Reiner reported that by one month of age, the Q140 mice displayed a shortfall in thalamostriatal axodendritic terminals. These included inputs to cholinergic interneurons, which had 40% fewer synaptic contacts than WT at one month. In addition, axospinous thalamo-cortical terminals were reduced by 20% by age four months at both direct- and indirect-pathway neurons, and that reduction was steady at 12 months. These findings indicate a possible developmental deficit in the formation of the thalamic input in these mice rather than a strictly progressive degeneration of the thalamic input. Direct-pathway neurons in particular saw a 30% decline in corticostriatal terminals by 12 months but not before. The loss of these terminals was correlated with phenotype severity in several measures of motor behavior. The implications of the findings may give clues about phenotypic progression of HD in humans, but the differences between human and mouse pathology and phenotype progression must be taken carefully into account (see Discussion Points).

Michael Levine from University of California, Los Angeles, presented a characterization of the interneurons found in striatum, and how their properties and connectivity were changed in the BAC-HD mouse compared to WT. “The interneurons set the tone of what happens in striatum,” Levine said. So we want to know whether they receive inputs differently in HD. In addition to several types of GABAergic interneurons, the striatum also contains large cholinergic interneurons (LCI), which also release glutamate, as shown in recent reports (Higgly et al. 2012). In R6/2 mice there was a decrease in somatic cross-sectional area in LCIs but no change in basic electrophysiology—until they looked at synaptic inputs. Spontaneous IPSCs recorded from

striatal MSNs were reduced in young R6/2 mice. At later ages, that finding reversed, and inhibitory inputs to MSNs increased in frequency. In contrast, the same experiments yielded the opposite results in BAC-HD mice: the inhibitory inputs to MSNs decreased in frequency over time. Graybiel wondered, How can we interpret these striking differences?

In an attempt to determine where the action potential-dependent inhibitory input arises, Levine considered the possibility that they arise at other MSNs within the striatum. In contrast to Faber's data, Levine reported that the inputs to MSNs from other MSNs had decreased in transgenic animals. Levine recorded from two striatal neurons and stimulated one while focusing on the post-synaptic potentials in the other—under conditions that all excitatory PSPs were blocked. There were fewer connected pairs of MSNs in the R6/2 mice at all ages tested. The amplitude of the evoked IPSPs was also reduced. The results were similar in both R6/2 and BAC-HD mice. In other experiments, Levine showed that MSNs from R6/2 mice displayed a bi-directional connectivity that was never seen in the WT striatum. Furthermore, while MSN-MSN connections seem to be regularly distributed among direct- and indirect-pathway neurons in WT, in the R6/2 Levine saw much more connectivity between direct-pathway MSNs. Finally, another class of interneurons called persistent low-threshold spiking (PLTS) spontaneously appeared to have stronger connections to MSNs in R6/2 mice. They fired more frequently and, in a small group of connected pairs, activation of PLTS interneurons evoked larger synaptic responses in R6/2 MSNs than in WT.

Jeff Macklis of Harvard University presented a potential new way of viewing HD as a neurodevelopmental rather than a neurodegenerative disease. Although the mutant huntingtin protein is expressed globally, the basal ganglia and the striatum in particular are preferentially vulnerable—at least according to pathology. Perhaps the vulnerability originates in a tiny subset of progenitor neurons, which then becomes a large, heterogeneous population within the basal ganglia and perhaps within the striatum itself. Macklis characterizes neurons as fundamentally heterogeneous; malleable cells that become shaped by events that are time-dependent, dose-dependent, stage-dependent and state-dependent. Each developmental step—such as turning on genes or responding to an external factor—occurs in a particular sequence, and differences in these events can fundamentally change the neuron and how it functions over its lifetime.

Macklis has so far concentrated his investigations on ALS and autism, and has primarily characterized cortico-spinal projection neurons. He plans to use the same approach to learn more about the cortico-striatal projection neurons critical in HD. He focused on the intra-telencephalic (IT) neurons with the following questions in mind: When and where are they born, and when and where do they send their axons? Do they stay stable? Do they undergo pruning, and where and when? His goal is to assemble an identity of molecular developmental controls and markers that might lend clues as to how and when these neurons deviate from "normal" in HD. Eventually, this signature might be used as markers of disease in mice and in humans. Ultimately, the goal would be to ask in human brain neuropathologically what's happening to these specific subsets of neurons. Are they dying, shrinking, or malfunctioning in disease? Ultimately Macklis hopes to address the question, why is a subset of function affected with a globally expressed mutant protein? Although the disease appears to affect adult neurons, perhaps the abnormalities arise during development.

Macklis suggests we reconsider neuronal heterogeneity, because any classification based on anatomy—even micro-structurally and even within progenitor populations —is too broad.

Macklis has carried out molecular and genetic comparisons of distinct neuronal types at various developmental stages. He has purified individual populations of neurons and then subtractively compared them, in effect "getting rid of the ten thousand genes that do the same thing in projection neurons." He has found specific subsets within the cortico-spinal and cortico-thalamic populations, each of which expresses about 25 specific genes. A few are transcription factors; others are axon- and synapse-guiding factors. Next, Macklis plans to carry out this specialized characterization within cortico-striatal neurons. Because previously described neuronal-specific factors and genes simply weren't specific enough, the new approach required that he use ultrasound-guided micro-injection of retrograde fluorescent label at each developmental stage to characterize the 5,500 cortico-spinal projection neurons found in mouse brain. Achieving this 99% purity in the sample of neurons in a population was of the utmost importance, he said, because the marker genes they identified to characterize subsets were "not expressed in brain" according to previous reports using large tissue samples from brain. In other words, one can't hope to find a unique signature or developmental chassis by grinding up a whole brain or even a brain structure when some of the genes are expressed in only 800 neurons.

Session 4: Astrocyte-Neuronal interactions in HD models

Lynn Raymond of the University of British Columbia discussed her findings on altered synaptic—and particularly extrasynaptic—NMDA receptor signaling in the YAC 128 mouse. Although synaptic NMDA signaling—even with intense stimulation—activates synaptic plasticity, activation of extrasynaptic NMDA receptors sets in motion alternate signaling pathways including shut-off of CREB and initiation of apoptosis in hippocampal and cortical cultures. Could this hold true in striatal neurons as well? If this deleterious signaling is increased in HD, it might partly explain the susceptibility of striatal neurons to dysfunction if not death. Raymond recorded from striatal projection neurons in WT and YAC 128 mice after treating with the drug TBOA, which blocks glutamate reuptake leading to increased extrasynaptic glutamate. The recordings revealed enhanced currents meditated by the GluN2B-type NMDA receptor subunit in YAC128 mice, and these extrasynaptic receptors were increased by 50% in YAC128 mice compared to WT at one month of age. She recapitulated these findings in MSNs co-cultured with cortical neurons. She used pharmacological manipulations to isolate extra-synaptic from synaptic NMDA-mediated currents and found that indeed they were increased in MSNs from YAC128 compared to WT mice. Nuclear p-CREB was inhibited to a greater degree after NMDA signaling in YAC128 mice, and apoptotic signaling was increased. Raymond also found that activation of the p38 apoptosis pathway required an association between the GluN2B subunit and post-synaptic density 95 (PSD95) as well as nitric oxide signaling. Surmeier and Levine reported that they too have seen increased extrasynaptic NMDA signaling, but preferentially in indirect-pathway neurons; however they were using a different slice preparation and stimulation. Macklis wondered why any neurons with NMDA receptors wouldn't also show this mis-distribution of GluN2B outside of synapses, and why in HD these neurons might be preferentially subject to harm through this mechanism when mHtt is globally expressed. Raymond pointed out that the developmental expression of GluN2B is such that in most neurons the subunit is not generally included in NMDA channels but in striatum expression remains high and enriched into adulthood. If other brain areas would not display the same distribution, they might be used as a point of comparison as to how it might arise.

Rosemarie Grantyn of Charité Berlin suggested that HD might be an imbalance in glutamate and GABA in the brain—that perhaps the excitatory-inhibitory balance is disrupted. Other results have shown that extracellular glutamate is elevated in R6/2 mice, perhaps indicating impaired clearance. In addition, in intact animals striatal neuron firing patterns are disrupted but can be somewhat normalized by ceftriaxone, a drug that increases GLT1 activity. Grantyn hypothesized that the astrocytic glutamate transporter GLT1 (EAAT2) might be a critical element in attempts to restore striatal GABA-glutamate balance. Grantyn gave special consideration to the slice preparation, with criteria including LFPs to show "the connectome is at least somewhat intact." In slices from R6/2 and Q140 mice, they recorded from striatal output neurons (SON, called MSN by others) and simultaneously from a nearby astrocyte in order to see GABAergic synapse-evoked astrocytic glutamate transporter currents. The paired-pulse protocol allowed them to make conclusions about release probability and plasticity. Indeed these transporter currents—and GLT1 function—were reduced in R62/ and Q140 mice; as a result, extracellular glutamate concentrations were tonically elevated around GABAergic synaptic terminals. Grantyn then demonstrated that synaptic GABA release was reduced at individual sites due to synaptic depression. That LTD was initiated at mGluR5 metabotropic glutamate receptors leading activation of presynaptic CB1 receptors. Grantyn asked next about parasynaptic GABA concentration and found evidence that it was reduced in the HD mice. In mutants, a tonic GABA(A)-type receptor-mediated chloride conductance was decreased, as was GABA(B)-type receptor-mediated presynaptic depression in SONs. Grantyn also presented preliminary evidence that the astrocytic GABA-transporter GAT-3 normally operates in "release mode," driven by the sodium influx generated by GLT-1 activity. She described an emerging hypothesis about GABA function in SONs: If GLT-1 dysfunction results in nonsynaptic GABA release from astrocytes, this could account for deficits 1) in synaptic GABA release (via the mGluR5-mediated depression) and 2) in the activation of extrasynaptic GABA(A)R-mediated tonic chloride conductance. These fundamental changes in transmitter and ion concentrations would carry functional consequences for the neurons' activity pattern and potentially their health and survival. Importantly, GABA still appears to function as an inhibitory neurotransmitter at SONs in HD mice, although the inhibition was weaker than that seen in WT mice.

Baljit Khakh of the University of California, Los Angeles, described some of the emerging thoughts about astrocytes and how integral they may be to synaptic function, including potentially releasing neurotransmitters themselves at synapses. When mutant huntingtin (mHtt) is expressed in glia alone (using the human GFAP promoter), an HD-like phenotype arises similar to other putative HD models, including low weight, scrawny appearance, clasping behavior, progressive motor deficits (rotarod performance), and early death. Interested parties should read this work from the Li lab at Emory University (Bradford et al. 2009) to assess how the reported phenotypes relate to other mouse models of HD.

Khakh showed experiments in which astrocytes in the standard R6/2 mice contain inclusions, but are generally not overtly reactive based on several standard markers, although some astrocytes showed signs of mild reactivity. Electrophysiological investigations of astrocytes revealed alterations in their membrane properties in R6/2; they were more depolarized and potassium currents through the Kir4.1 channel were reduced. Using viral delivery of a calcium indicator tethered to cell membranes, Khakh was able to visualize the finely branched astrocyte processes, which are elaborate and far-reaching beyond the cell body and major processes. He likened the image of the visualized "branchlets" to the snow-covered fine branches of an oak tree photographed by Ansel Adams. He pointed out that the relevant domain of astrocyte is the

fine branches, and we need to develop more tools to investigate there. Even in GFAP-negative astrocytes, the GFAP promoter worked to drive the viral gene expression. Khakh next showed spontaneous calcium waves in astrocyte processes, which he described as a self-propagating slow-wave movement, suggesting it could have a function in cell communication, and may be driven in part by neuronal glutamate.

Next Khakh described his hypothesis of what might contribute to astrocyte dysfunction in HD, which he called the "double-potassium punch." The first punch comes with the change in potassium, and how it affects a mechanism called potassium siphoning. Arising from local differences in activity and membrane potential, cells siphon potassium away from areas of high activity to areas of low activity. He predicts that the elevated potassium seen in the R6/2 would compromise this potassium-siphoning process. In the second punch, Khakh believes that the GLT1 malfunction might in fact arise from the demonstrated deficit in Kir4.1 potassium channels. With the membrane and reversal potential changes that arise from the channel loss, glutamate uptake would be compromised because GLT1 transport is voltage-dependent. An extracellular potassium concentration of 50 mM would result in glutamate extrusion through GLT1—an unlikely scenario—but even if it reached only 5 or 10 mM, glutamate uptake would be compromised. Khakh plans to test this hypothesis using viral delivery of Kir4.1-GFP fusions in R6/2 mice, which he predicts would rescue the 5mV change and with it possibly GLT-1 expression and glutamate uptake. He wants to determine from postmortem tissue whether Kir4.1 is reduced in human HD brain.

Session 5: Dopaminergic status in HD; therapeutic strategies

Kathy Toreson working in the lab of Patricio O'Donnell at the University of Maryland has become an expert in recording dopaminergic neurons in multiple systems, and her focus will be to home in on these neurons in the transgenic BAC-HD rat (tg5). In HD patients, postmortem brains have decreased tyrosine hydroxylase (TH)-positive neurons, and the remaining dopaminergic cells are smaller and express less TH than normal. Tg5 rats have a reduced brain volume compared to WT at 12 and 15 months according to MRI. Toreson explained her experimental set-up. The orbitofrontal cortex (OFC) doesn't encode information about a reward itself, but about the expectation of a reward. She has measured in Long-Evans rats whether the OFC can modulate DA neuron firing. In anaesthetized rats, she stimulated the OFC and recorded from DA cells in ventral tegmental area (VTA). Juxtacellular recordings allow for stable data collection and labeling of the cell for positive identification as dopaminergic. Measures of baseline firing rate, action potential (AP) duration and instantaneous firing frequency matched with what's reported in the literature. DA cells showed a characteristic inhibitory response to OFC stimulation. Toreson presented very preliminary results from four animals describing the physiological properties of putative dopaminergic DA neurons in the transgenic fragment tg5 rat. In WT Sprague-Dawley rats at 6 months and in tg5 rats (also Sprague-Dawley), the baseline firing rate and AP duration resembled the Long-Evans rats, but instantaneous firing frequency was altered in both WT and tg5. Toreson aims to further assess the firing patterns in the HD model DA neurons, and to measure their response to stimulation of the medial prefrontal cortex (PFC) and OFC. Her goal is to record at three, six, nine, and 12 months, and see whether alterations arise between tg5 and WT, and when.

Roger Cachope from University of Maryland - Laboratory of Joe Cheer, highlighted "the complex and profound arrangement of cognitive alterations" seen in HD patients. He plans to compare WT and BAC-HD (TG5, 97 repeats) rats in a longitudinal study to determine how specific striatal output changes develop with age in the HD model and how they might be affected by prefronto-cortical and VTA inputs. Voltammetric recordings of striatal dopamine levels will be performed on anesthetized animals. More important, either multiple-electrode electrophysiological recordings from prefrontal cortex and striatum, or striatal dopamine levels will also be monitored in freely-moving animals while performing on behavioral tests for motivation. Cachope described fast cyclic voltammetry as the technique of choice to measure real-time striatal dopamine levels. They implant a small 7-micron carbon fiber into striatum and simultaneously pass a triangular wave of voltage, taking advantage of an electrochemical reaction by dopamine. At one specific voltage potential it gets oxidized and at another one reduced—both contained within the waveform. The oxidation-reduction reactions generate a small but detectable current, producing a signature of DA levels in the region. Cachope reported preliminary results on ventral striatum dopamine levels from anesthetized in vivo animals. As yet with the small number of animals examined, no differences have emerged between WT and TG5 rats at six months of age, but they expect to find differences between genotypes when studying them at later ages, up to one year.

Geoff Tombaugh of PsychoGenics has begun to characterize the electrophysiological properties of striatal MSNs from R6/2 mice. Ultimately, he hopes that these signatures might represent assayable biomarkers that might be altered by treatment, and could be used as drug screening criteria. One such arena is the kynurenine pathway. KMO (kynurenine monooxygenase) inhibitors have been a heavily invested target area at CHDI and were recently shown to attenuate mortality and loss of cellular markers in striatum of R6/2 mice. While most studies of electrophysiological properties measure synaptic changes, Tombaugh has focused here on potentially useful non-synaptic passive and active membrane properties throughout disease progression. The most striking difference he sees is a higher input resistance (R_{in}) and consequently a lower rheobase, resulting in a slightly hyper-excitable neuron. He has also seen a slightly reduced AP amplitude and increased AP threshold. The KMO inhibitor reversed those excitability changes in R6/2 mice after acute application to a para-horizontal slice. Next he aimed to show a change in the disease progression by chronic treatment with the drug, but systemic delivery in the animal (followed by 1-2 days of washout) did not rectify the membrane properties in R6/2 mice as the acute treatment had done.

Christopher Schmidt of Pfizer did not make his presentation, as many participants had recently seen it at CHDI's Palm Springs meeting in February.

Discussion Points

Jeff Macklis asked why, in HD and in many other neurodegenerative diseases (NDD), would we think that an abnormality would happen in a large brain area rather than in a distinct cell type arising from a particular neuronal lineage? During Surmeier's and Bevan's talks Macklis asked whether tests were done on "chunk" of cortex or on highly specific, separated neuronal populations. He advocated that we study the specialized subsets of neurons within each basal ganglia structure. The research presented here may indeed be describing these subsets' distinct

electrophysiological properties and sensitivities to CAG expansion in the huntingtin gene. Also during Zhong's talk, Macklis pointed out that there are likely many subtly different subsets of neurons throughout the basal ganglia structures, each with distinct circuitry, electrical behavior, and gene expression profiles.

Participants at several points discussed which brain areas should receive priority in future studies based on animal and human data about disease progression. For example, Levin reported her recordings in "mouse PFC." But according to human MRI data, that area of cortex is not apparently affected until later in the disease course. The HD-related cortical thinning seen in patients arises more posteriorly. (Although Graybiel points out, these findings depend on the patient subgroup; see Richard Faull's work on New Zealand HD postmortem brain samples). In prodromal HD, PFC is the most preserved area, and therefore least predictive of onset of disease, even after pronounced, quantifiable cognitive deficits develop. It might make sense for Galenea to measure LFPs in mouse striatum. There is the caveat that this human data reflects pathology—i.e. gross morphological changes. Rebec pointed out that HD does give rise to significant cognitive deficits, so one might expect PFC changes even despite a lack of gross pathology. Additionally, even within the HD patient population, pathology can be quite heterogeneous, as is the onset of cognitive deficits. Still, the information can be used as a rough guide. Tobin appealed to scientists to select circuits to probe based on "what has already been studied with behavior in humans and connect it to human reality." People studying early and prodromal HD have now produced 15 years of data, so we need to consider that, even if you reject it. He implored participants to design experiments based on data, not on what's the most convenient model in animals.

Vahri Beaumont summed up the background regarding the direct- and indirect-pathway neurons in HD patients and in transgenic animals. In HD patients, the classic motor symptom is "hyperkinesis." In addition, the indirect-pathway neurons (which express D2 dopamine receptors and substance P) are—pathologically—more vulnerable early in HD compared to direct-pathway neurons (which express D1 receptors and enkephalin). The circuitry is such that the loss of indirect pathway activity is thought to remove the "brake" on basal ganglia output, resulting in hyper-kinesis.

In all the mouse and rat models of HD, in contrast to humans, the motor phenotype in frank disease is hypokinetic (however, see below). It must be noted that the transgenic models use a very long CAG-repeat length. Could it be possible that the animal models might more closely recapitulate the juvenile form of HD? In these patients, the kinetic symptoms appear different and more Parkinsonian than in adult HD. The phenotype in the HD models might arise from simultaneous progressive loss of both the direct and indirect-pathway neurons, in which case both the "brake" and the "gas" of basal ganglia activity declines. Graybiel and others said this idea warrants further investigation.

With respect to the animal motor phenotype, Levine described a transient motor behavior he described as vertical hyperactivity; "They rear more," he said. That's seen in many models around two months, and then it gives way to the hypokinetic phenotype seen across all rodent models, which he described as less movement in an open field. Raymond pointed out that even in human patients who are newly diagnosed with HD, up to 50% of the striatal volume is already gone. Humans display gross pathology before motor symptoms arise. Participants agreed that neurodegeneration is and should be less of a focus than neuronal function; how can we improve

the efficacy of basal ganglia signaling while the players are intact? The nature of the striatal volume loss remains unclear; myelin, glia, axons, and other neuropil components may be preferentially lost. Astrocytes have certainly emerged as potential players in HD. Raymond's point was also that subtle differences in the firing properties of striatal neurons are unlikely to underlie motor symptoms because of the massive structural change that occurs even in relatively early stages of the disease. However, other types of symptoms (cognitive and emotional) might be linked to these more subtle changes in connectivity or electrophysiology. The charge at hand seems to be to find the very early, as-yet-undetected deficit that arises in humans from striatal neuron dysfunction while they're still intact.

Another discussion topic that surfaced several times was the issue of weight in transgenic rats. Whereas human patients lose weight, the transgenic rats become obese, an effect that seems to be specific to BAC HD. In work from Åsa Peterson, when mHtt was removed from the periventricular hypothalamus, rats' weight was normalized. The overweight may be specific to the strain of rat; even wildtype rats get very large according to some participants.

Participants	Institution	Experimental Preparation
George Rebec	Indiana University	extracellular unit recording and LFPs from behaving transgenic mice
Anthony West	Rosalind Franklin University	*in vivo* recordings from MSNs in behaving BAC-HD transgenic rat
Margaret Levin	Galenea	LFPs from association cortex in behaving transgenic mice (3 models)
Anne Graybiel	MIT	
Donald Faber	Albert Einstein College of Medicine	R6/2 mice
James Surmeier	Northwestern University	Caged glutamate; MSNs in R6/2 and Q175 mice
Mark Bevan	Northwestern University	Ex vivo slices from GPe and STN of R6/2 and Q175 mice
Sheng Zhong	PsychoGenics	Single-unit recordings from STN and GP in anesthetized BAC-HD rats
Anton Reiner	University of Tennessee Health Science Center	Cortico-striatal synapses in Q140 mice
Michael Levine	University of California, Los Angeles	Interneurons and MSNs from R6/2 mice
Jeff Macklis	Harvard University	Molecular characeterizatin and tracking of cortico-spinal projection neurons
Lynn Raymond	University of British Columbia	Striatal projection neurons from YAC 128 knock-in mice
Rosemarie Grantyn	Charité Berlin	MSNs and astrocytes from slices in R6/2 and Q140 mice

Baljit Khakh	University of California, Los Angeles	Calcium waves from astrocyte processes from R6/2 mice
Kathy Toreson, Patricio O'Donnell lab	University of Maryland	DA cells in ventral tegmental areas (VTA) in tg5 rats
Roger Cachope, Joe Cheer lab	University of Maryland	Fast cyclic volatmetry of DA cells in ventral tegmental areas (VTA) in tg5 rats
Geoffrey Tombaugh	PsychoGenics	Recording from MSNs from R6/2 mice
Christopher Schmidt	Pfizer	

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