CHDI Workshop: Basal Ganglia Electrophysiology—Sep 23 & 24, 2008
Report prepared by Stephani Sutherland Ph.D., for CHDI

- Executive summary: overview of report

- Introduction to Huntington's Disease and the Basal Ganglia

- BG Circuitry and Neuronal Profiles: This provides a more detailed overview of the cells and structures contained within the BG and their interconnections, with a focus on a more extensive characterization of medium spiny neurons (MSN) of the striatum. The information builds on the basic "Box Model" understanding of BG but includes updated details provided by workshop participants during discussions. This section provides valuable context for the Workshop Insights that follow.

- Workshop Insights: This section provides key details from the workshop discussions. The insights include some experimental results (published or unpublished) from workshop participants, identified by their initials, and some data from work by non-attendees, identified by name. The section is organized into the following subsections:
    - Cellular Physiology
    - BG Circuitry
    - Clinical insights
    - Imaging

- Big Questions: This section summarizes the "take-home messages" distilled by participants mostly at the close of the workshop. Though the goals are far-reaching and may seem daunting, they contain many immediately answerable questions that could yield key insights to understanding HD. Central questions revolved around the time course of HD, determining primary vs. adaptive effects, and the identity of the initial synaptic defect.

- Experiment alerts: These suggestions for very specific experiments were outlined by participants at various times throughout workshop discussions and have been compiled in this separate section.

- Appendix: Experimental Considerations. This section contains more technical considerations for researchers working in the field of HD, including discussions about animal models, brain slice preparations, and pharmacological issues.

- Chronological Highlights: This separately available document contains a chronological documentation of key discussion points as recorded directly during the workshop, distilled from written notes and audio files. This document contains more specific information about individual participants' input.

Introduction to Huntington's Disease and the Basal Ganglia

Huntington's Disease (HD) is a genetic neurological disease with autosomal dominant inheritance. HD was originally termed "Huntington's Chorea" for the characteristic jerky, uncontrolled movements seen in patients. We now know that the effects of HD extend far beyond simple movement disruptions to include cognitive and mood symptoms, which arise much earlier. In fact, many patients report these symptoms as more debilitating than movement problems.
HD is caused by a mutation in a single gene, htt, which codes for a protein called huntingtin. In the mutant form of htt, the gene contains a repeated code for glutamine—one of the 20 amino acids that make up all proteins. HD is one of several neurological disorders called "polyglutamine diseases." The number of "glutamine repeats" contained in the htt gene influences the disease's time of onset and severity in the patient. Despite these advancements, our understanding of how mutant huntingtin affects neurons and circuits within the brain is limited.

Huntington's disease (HD) and other movement disorders affect a group of brain nuclei called the basal ganglia (BG) and the cortex. (The cortex is a highly organized, complex structure that interacts with nearly every other part of the brain. It is considered separate from the BG.) A simple "box model" has served to provide a basic understanding of the structures of the BG and their interconnections, but these connections are complex and dynamic.
At the center of the BG is the striatum, and this structure is the primary target of HD pathology. Within the striatum, medium-sized spiny neurons (MSN) receive and send communications from and to the cortex and the other parts of the BG. In the HD brain, these connections are disrupted and these neurons eventually die. MSNs receive excitatory input from the cortex and the thalamus in the form of the neurotransmitter glutamate (Glu), and they receive the excitatory neurotransmitter dopamine (DA) from neurons of the substantia nigra pars compacta (SNc). MSNs are also influenced by interneurons within the striatum that deliver the inhibitory neurotransmitter GABA. These interneurons in turn are directly influenced by inhibitory neurons of the globus pallidus (GP).
The output of the MSNs is organized into two main pathways. In the direct pathway, MSNs send signals to neurons of the substantia nigra pars reticulata (SNr). This nucleus then sends signals downstream to the thalamus and other nuclei, which in turn send feedback to the cortex. In the indirect pathway, MSNs send projections first to the GP, and these signals are then sent downstream to the sub-thalamic nucleus (STN) and the SNr.
If this sounds complicated, be aware that it is an extremely simplified overview. Our understanding of normal BG function is far from complete, and our inquiry into HD's effects has just begun. But questions have now been identified—many of which are immediately answerable—that can help advance us towards better treatments for HD.

- **Basal Ganglia Circuitry and Neuronal Profiles**

Within the BG, the structure most affected by HD—and the area that has received the most research attention—is the striatum. Within the striatum, medium-sized spiny neurons (MSN) make up over 90% of the neurons. HD results in the death of these cells.

MSNs form two segregated populations, which can be differentiated according to their projection pattern (to downstream targets outside the striatum) and their dopamine (DA) receptor expression.

1- Direct pathway neurons
    - Project “directly” to the output nuclei of the BG via SNr
    - Express the D1-type DA receptor
    - Have a larger, more spread-out dendritic tree
    - D1Rs control induction of long-term potentiation (LTP)

2- Indirect pathway neurons
    - Project to an “indirect” downstream target, the GP
    - Express the D2-type DA receptor
    - Are smaller, with fewer dendrites and a smaller surface area
    - Are preferentially affected sooner in HD pathology than direct-pathway MSNs
    - Have stronger recurrent collateral networks than D1 neurons
    - LTP induction controlled by expression of A2A-type adenosine receptors

The remaining 10% of striatal neurons make up several classes of interneurons. These include the fast-spiking (f-s) GABAergic interneurons, which can be identified by their immunoreactivity to parvalbumin. These f-s interneurons receive massive excitatory inputs from the cortex and probably the thalamus, as well as inhibitory inputs from the external segment of the globus pallidus (GPe). It’s not clear whether they receive input from other striatal interneurons. Their primary target is the MSNs; one individual f-s interneuron can control hundreds of MSNs. They are minimally active, but their synchronization may determine which MSNs are active at any given time. There are few reports focused on HD’s effects on these interneurons. Another subset of striatal interneurons is cholinergic.

Connections between neurons of the BG include but are not limited to:
- Cortico-striatal connections (glutamatergic)
- Thalamo-striatal connections (glutamatergic): Substantial, but little research has examined them. Thalamic neurons also send inputs to

cortex and sub-thalamic nucleus (STN). These inputs are characterized as complex, primary inputs, not just feedback.

- Cortico-cortical connections: (both of these cell types innervate MSNs)
    - Intertelencephalic systems between pyramidal neurons with strong collateral projections
    - Cortico-fugal systems with some minor local axon collaterals

  Note: HD seems to affect cortical cells indiscriminately in that they all develop mutant HTT inclusions and cortical cell death appears to be generalized.
- Globus pallidus (GP)
    - One quarter to one third of GPe neurons specifically target striatal f-s interneurons, which in turn influence MSN activity. GPe neurons are poised to exert tight control over striatum.
    - GPe sends projections to GPi, SNr, STN and SNc. GPe activity may be central to BG; connections throughout BG are complex, and do not simply convert the sign of an incoming signal. GP neurons are tonically active and GABAergic.
    - GP and STN are richly interconnected inhibitory and excitatory neurons, respectively. In PD (and possibly in HD), these cells oscillate. In wild-type brain, several factors de-correlate their activity to prevent oscillation, namely autonomous pacemaker spiking at ~40 Hz by both neuron types.
    - Extensive collaterals within the GP itself
- STN sends glutamatergic inputs to GP and SNr
- SNc sends dopaminergic inputs to MSNs of the striatum

MSNs are characterized as “engineered to be quiet.” They sit at a naturally low resting membrane potential (RMP) far from the voltage threshold required for action potential spiking. They also have an input resistance that causes them to resist excitation, partly due to their expression of inward-rectifying (IR) potassium (K) channels. MSNs are much less excitable than are cortical neurons, which have an RMP closer to spike threshold. Within the striatum, one third of MSNs (in rat) are electrically coupled by developmentally regulated gap junctions.

Because they are engineered to be quiet, MSNs require highly correlated signals in order to fire. They receive massively converging inputs from many areas of the cortex and from the thalamus. In the context of HD, MSNs become hyper-excitable, shed synapses, lose spines, and eventually die. Their progressive decrease in connectivity with the cortex (and possibly with other areas) might be an attempt to restore their activity back to some target level. It’s unclear what signals they might be listening to during this process of disconnection. When they lose those highly coordinated inputs, it becomes nearly impossible for MSNs to ever be activated at the right time, which has unknown behavioral consequences for movement.

MSNs (and other neuron types) can exist in an “upstate,” in which they are more receptive to inputs and more apt to fire, or a “downstate,” in which they are less receptive to inputs and quieter. In anesthetized animals, neurons spend much more time in the downstate but do make some transitions; in awake animals, MSNs spend more time in the upstate.

Workshop insights: The following section provides key details from the workshop discussions. The insights include some (published or unpublished) experimental results from workshop participants, identified by their initials, and some data from works by non-attendees, identified by name. The section contains the following subsections: Cellular Physiology, BG Circuitry, Clinical insights, and Imaging.

Cellular Physiology

- JS has seen developmental regulation of input resistance in MSNs; by 6 weeks, D1 and D2 neurons differed by about 20-30%. An upcoming paper in *J. Neuroscience* details the reconstruction of dendritic trees in D1 (more spread-out) and D2 neurons (more compact).

- JS: Strategies to find molecular fingerprints of D1 and D2 neurons include gene array experiments. Early results have yielded long lists of genes that differ between the two cell types, including phosphodiesterase (PDE). This strategy may be important for identifying sources of D2 neuron vulnerability.

- Calcium homeostasis is certainly an important factor in HD pathology. Early stages of homeostasis disruption are potentially far more interesting than late-stage classic “excitotoxicity.” These early-stage changes may underlie altered cognitive, mood, and motor functions. Disruption may have broad effects on cell activity and connectivity regardless of whether the cells die.

- Voltage-gated calcium channels (VGCC) play a pivotal role in neuronal excitability and calcium homeostasis. They may provide a valuable target for HD therapeutics, and certainly provide a good target for better understanding of cellular physiological processes. Good genetic and pharmacological tools are immediately available for experiments to probe VGCCs.

    - The inositol triphosphate receptor (IP3R) is dysregulated in animal models of HD. NR2B-type NMDA glutamate receptors are upregulated in HD (compared to wild-type). VGCCs are physically located next to NMDARs, and they have a critical link to IP3Rs. They’re synaptically located and control plasticity. These factors make VGCCs a likely player in HD synaptic pathology.

- Particular classes of VGCCs shape synaptic responses in MSN dendrites. These channels may underlie Ca dysregulation. JS: in distal parts of MSNs, two channels in particular may be important. A known drug targets one of them; the other could probably be targeted for small-molecule screening. If pathological synaptic responses and or disconnection result from calcium entry through those channels (whether the problem results from loss of channel activity or over-activity), that provides a short path to a therapeutic drug.

- Another possibility is that IP3 receptors, which are directly linked to the calcium channels, may be dysregulated, resulting in elevated intra-spine calcium concentration that triggers homeostatic machinery to eliminate the synapse. That could be prevented.

- A2A-type adenosine receptors are key to induction of LTP in the indirect pathway, and they represent a potential target for both therapeutic and inquiry tools.

    - AK: At indirect pathway (D2) MSN synapses, A2AR is expressed postsynaptically (at MSN dendrites), but there is no evidence for presynaptic expression. At direct pathway synapses, there is evidence for both presynaptic and postsynaptic expression of A2AR. JS: mRNA levels for A2A are very low in cortex compared to striatum, so postsynaptic A2A should be the focus.

    - There is some evidence (from Raphael Franco at NIDA) that the affinity of A2AR antagonists differs at A2A-D2 heteromers vs. A2A-A1 heteromer complexes. (A2A-D2 describes an intra-membrane interaction—possibly a heteromerization—between A2AR subunits and D2-type DA receptor subunits.) Caffeine does not have equal affinity at these receptor types. Whether these A2A-D2 heteromers form in native neurons or not, the affinity should be considered in development of any small molecule. Because the expression level is so much greater postsynaptically than presynaptically, it may not matter functionally in an animal.

    - HD patients express less A2A receptor transcript than normal—possibly simply because of dendrite and synapse loss. Because A2A leads to LTP in D2 neurons, this could translate to less potentiation of the indirect pathway in HD.

    - RC: By delivering A2AR antagonist, you may be decreasing firing or potentiation further. The result is hyperactivity, as expected. But what if you delivered A2AR agonist? Some experiments have looked at this

with minimal effects, but these agonists have not been BBB-penetrable.

- What are the possible sources of adenosine in the striatum? If ATP (anionic) were co-packaged with a neurotransmitter, the best candidates would be cationic molecules like DA or ACh. Striatal cholinergic interneurons may be a source.

- ES experiments compared upstate vs. downstate of MSNs in R62 HD model vs. wild-type (anesthetized) mice. The time spent in the two states was similar between animals, but the pattern of transitions between the two states differed: in HD mice, it was easier to evoke state transitions from down- to upstate and from upstate to firing. The amplitude of responses in either state did not differ between wild-type and HD mice.

- ES: In Alzheimer's disease (AD), cells develop plaques similar to inclusions seen in HD. Even in very advanced-age mice, with removal of AD pathology, neurons can recover from horrible morphological adaptations. The early diagnosis and predictability of HD would allow for earlier treatment, but treatments may provide benefits even for advanced disease.

BG Circuitry

- CC describes experiments in slices from HD animal models. Their most important observation was a progressive disconnect between the cortex and the striatum at age 4-6 weeks that worsened with time. By 15 weeks, no EPSCs were detected, indicating a loss of inputs to striatum. Around 5-7 weeks, they observed very large spontaneous events, some involving a calcium after-discharge. Their interpretation was that cortical hyper-excitability resulted in increased glutamate (Glu) release in the cortico-striatal pathway. Although the primary insult may have been cortical, in the form of dysregulated Glu release, the result was loss of striatal MSN spines, synaptophysin, and synapses. They believe those inputs arose from cortex rather than the thalamus or elsewhere, because those spontaneous events were lost after removal of the overlying cortex. Even in animal models that don't display cell death, cortico-striatal synapses diminish and spines are lost.

- Cortical and thalamic neurons form synapses onto striatal MSN dendritic spines, where they release glutamate. The presynaptic neurons express vesicular glutamate transporters (vGluT) to package glutamate into vesicles for synaptic release. Cortical neurons express vGluT1, and thalamic neurons express vGluT2. This differential expression could be used to determine whether cortico-striatal or thalamo-striatal synapses are preferentially affected by HD.

- New technology allows for the manipulation of neurons *in vivo* using combinations of genetic, chemical and optic techniques. This technology may be useful in exploring the neurons and circuits affected by HD. The basic premise is that a protein is either expressed or tagged to make neurons sensitive to manipulation by a chemical agonist or to light. The three main examples are as follows:
    - The TRPV1 channel passes inward, excitatory current, is normally expressed in sensory neurons that transmit sensations of pain and temperature, and is activated by capsaicin, the active ingredient in hot chilies. Central neurons don't normally express the protein, but transgenic expression would make neurons sensitive to capsaicin and could allow for activation or even excitatory ablation of selected neurons.
    - Tagging of potassium (K) channels with a label that makes them light sensitive could allow one to permanently or temporarily disable K channels with a fiber optic delivery of light. Loss of K currents would result in neuronal excitation and, eventually, excitotoxic cell death.
    - Other applications include transgenic expression of (light-sensitive) rhodopsin proteins or of the diptheria toxin receptor, which is not normally expressed in mouse brain. The challenges associated with these techniques lie mainly in delivery of either the agonist (diptheria, capsaicin) or light (using a fiber optic) to deep-brain structures.

- Group discussions yielded the following consensus regarding these technologies:
    - Light is a better option than chemical agonists (if you can deliver to the proper neurons) because of rapid delivery and tight temporal and spatial control. RC maintains that reaching deep structures with fiber optic should not be difficult; they have used that technique in mice.
    - Ablation studies may not be the best option for understanding cellular effects, but could be a valuable tool for looking at behavior *in vivo* with temporal control.
    - Other possibilities would be to simply manipulate the activity of particular neurons without killing them, e.g. reduce activity of hyperactive cortical neurons that form synapses with MSNs. You would first need to identify whether the initial pathological change occurs presynaptically or postsynaptically.
    - RC suggests possibility of long-term delivery of light, not as an exploratory tool but to silence an identified pathway.

- William Yang: when mutant HTT was expressed only in cortex or only in striatum, symptoms and histological changes were reduced compared to global expression. This data supports the idea that cell-cell interactions are important. The data did not distinguish effects at direct- vs. indirect-pathway MSNs.

- George Rebec paper: Recorded from MSNs of two HD models: R6/2 mice and knock-in (KI) mice. Compared to wild-type, overall firing rate was elevated in R6/2 but not KI mice. In both models, burst activity was altered and recordings from pairs of MSNs showed that correlated firing and coincident bursts were decreased in HD, indicating that coordinated MSN activity was dysregulated. Attendees outlined two possible explanations: 1) increased postsynaptic responsiveness to a decreased number of pruned inputs resulted in synchronous firing between neighboring neurons—which the system is normally engineered to avoid at all costs. 2) MSNs are connected by recurrent collaterals, which promotes synchrony, particularly between D2 MSNs.

- JS: GP and STN are richly interconnected inhibitory and excitatory neurons. In Parkinson's disease (PD), and possibly in HD, those cells oscillate. Deep-brain stimulation (DBS) eliminates that oscillatory activity. Autonomous spiking at 40 Hz is thought to de-correlate their activity and prevent oscillation in normal brain. In PD models, this spiking is lost from GPe neurons. The molecular mechanism appears to arise from downregulation of a single gene, which can be re-introduced, and hopefully (but has not yet been shown) restore normal activity. Thus, the GP may prove to be a valuable target for HD as well. Two points to consider are: 1) corrections in the striatum may not affect this GP-STN circuit, and 2) motor symptoms might be resolved by manipulation of GP without touching striatum.

- Experiments measuring saccadic eye movements (in primates) can be used to study how voluntary and involuntary movements are the domain of various aspects of reward, learning, and movement-planning pathways. According to the conventional model, actions elicited by positive reinforcement engage the direct pathway MSNs, whereas suppression of punished acts engages the indirect pathway MSNs. RC speculates that direct pathway underlies voluntary movements; indirect pathway is more involved in involuntary movements.

- The mammalian striatum is organized as a mosaic of two compartments: matrix and patch (or striosome). Cells can be differentiated according to immunoreactivity in the two compartments. Discussion touched only briefly on this organization; these may be affected differentially by HD.

Clinical insights:

- Although HD was first classified as a movement disorder, our understanding of HD has evolved beyond that to include many cognitive and mood disturbances. A major goal should now be to identify pre-symptomatic readouts of the disease.

- HD has been called a “gain-of-function” movement disorder. Choreic movements must arise from underlying neurological dysfunction, perhaps because neurons are synchronously firing at the wrong time. That activity may be either absent or suppressed in the wild-type state. The disease state may represent too much synchronous activity or a loss of inhibition. The same neurological basis may underlie other, non-movement symptoms.

- Clinical symptoms of HD first appear as mood and cognitive effects, later followed by motor symptoms. This correlates topographically with a spatial gradient in the BG: areas are affected in a rostro-caudal gradient. A major goal is to find clinical markers or morphological indicators of this progression in human patients.

- Most of the emphasis in understanding HD in the clinical setting has focused on the basal ganglia (BG), and not enough energy has gone into understanding the cortical features. Also, little is known about effects of HD in the thalamus, except that the cholinergic neurons located there are spared.

- Juvenile-onset HD presents differently from adult-onset HD. Normally, adult patients display hyperkinesis with uncontrolled movements, whereas juvenile patients look more like end-stage HD patients or like Parkinson’s disease (PD) patients, with a more akinetic phenotype. This may correlate to a greater, earlier effect on the indirect pathway MSNs.

- Deep-brain stimulation (DBS) may benefit HD patients. DBS studies validate the idea that GP presents a valuable target in HD. DBS affects primarily motor symptoms (and is targeted to areas of GP controlling movement) and either does not affect cognitive symptoms or creates negative cognitive side effects. DBS has no effect on dementia, and can improve sleep.

- It’s unclear exactly what (electrophysiologically) DBS accomplishes. Although the input/output “box” model of the BG circuitry is a static model, all networks are dynamic, and the pattern of their activity is crucial. DBS imposes a rhythmic background and eliminates the aberrant patterns imposed by disease states.

- Cessation of DBS in PD patients results in return of symptoms. DBS treatment normally continues throughout a patient’s life. It’s unknown whether DBS slows disease progression and degeneration, or simply relieves symptoms.

- The threshold for seeing symptomatic changes (whether adaptive or primary) may differ in various areas of the brain. Compensation in different brain areas may take different forms.

Imaging: (input mostly from BD)

- Even before motor symptoms surface, striatal volume decreases, probably due to loss of dendritic trees, increased density, and possibly loss of cells.

- Some evidence has shown that HD patients show cortical thinning in imaging studies, but several attendees expressed skepticism about the reliability of such readouts. Also, it's difficult to isolate cortical effects morphologically because the affected circuitry is part of a loop with the BG. More reliable are data showing reduction in the size of the head of the caudate and the ventral striatum.

- MRI and multi-spectral methods of imaging may provide good insights. One should not use PET methods when non-invasive alternatives are available without radioactive drugs.

- We can now study both structure and function of the BG with imaging techniques. Until now, about half of the structures of the BG, including the pallidum, SN, and STN could not be visualized due to limitations in the signal-to-noise ratio. In labeling procedures, you need certain contrast between gray and white, and because of the histological features of these structures, they don't have good contrast like cortex does.

- There have been many different approaches toward studying structure. Out of our need to show results, we have been restricted to certain regions of interest, which produces bias. We need to determine where changes first occur in HD. We need to take a whole-brain approach. We now know that almost the whole prefrontal, motor and striatal regions are involved in the pathology; it's difficult to restrict efforts to motor cortex and its interactions with the BG.

- Functional studies are limited, both in normal and BG disease subjects. The issues are: 1) localization within a particular structure, and 2) having the machinery to study functional relationships between different nodes. This is new technology, and there are few people using it. The goal is to see how interactions between different nodes behave in simple fMRI experiments where first healthy then HD patients perform motor tasks. We want to get activation of those particular nodes, then, using this new machinery, see where the interaction is and how it differs between normal people and HD patients. This is essential; there are no studies considering different nodes of different models and comparing them. This would all be using BOLD (blood oxygen level-dependent) activity: an increase in oxygen flow and blood flow indicates increased synaptic neuroactivity.

- Diffusion tensor imaging is also interesting, but of course there are pitfalls, and in-depth anatomy knowledge is important.

- There are two main methods of studying connectivity in brain: 1) try to connect a and b, then come up with some probability that they're related; this is very variable; 2) a better technique for HD: compare topography of cortex and BG connections. If you assume there are certain connectivity problems, even if we can't measure the strength of these connections, we should at least see dynamic change over time in the topography of these connections.

- Developmental aspects of HD might benefit from imaging studies in children, possibly doing longitudinal studies.

- Best strategy may be to spread research around among small projects with different techniques. For example, ask people doing functional MRI studies to try looking at HD patients. Goal is to prove concepts, then take that data and correlate functional and structural changes using electrophysiology or tracing studies.

Big Questions: This section contains the overall, take-away questions that came out of the discussions, most of which were distilled by participants in the closing session.

- Circuitry of functional (wild-type) BG

We require a better understanding overall of BG circuitry, which might lead to strategies to manipulate output of BG in HD. Many attendees were surprised to learn how limited our current understanding is of BG circuitry. The BG is extremely complex, and it simply requires more research attention. The group's estimates of a time frame for a complete understanding of BG circuitry ranged from two to 20 years. Although the task seems daunting, many experiments could be done immediately. Specific goals include the following:

  - identify all neuronal components of the circuits and their dynamic patterns of activity
  - determine how these circuits dictate motor behavior
  - determine how alterations lead to motor dysfunction in disease.

- Time course of HD—which changes are primary and which are adaptive?

This key question runs through every area of discussion. HD is a temporally dynamic disease. Some changes (in synaptic physiology, in circuitry, in behavioral symptoms) may be primary HD defects (due to mutant HTT expression), whereas others are certainly adaptions to changes elsewhere. What are the very initial primary events?

Because the changes in HD occur over a lifetime, some view it as a developmental disease. At the cellular level, synaptic alteration is evident very early. It's important to look at various time points in the disease, particularly at the pre-symptomatic stage. Examination of HD at the earliest stages is most

likely to reveal changes and deficits that represent initial dysfunctional steps in disease, not just compensations or homeostatic adaptations. There is an immediate need to find earlier, pre-symptomatic readouts of HD, in cellular as well as systems and clinical settings. In order to better understand the disease time course, experiments should be temporally correlated, including behavior, *in vivo* work, slice physiology, and cellular physiology.

- Dysfunctional synapses at MSNs in the striatum:

The biggest clue to understanding HD lies in the striatum. Within striatum, HD clearly targets MSNs. The cortico-striatal (and or thalamo-striatal) glutamatergic synapse needs to be mechanistically pursued. Dendrites of the MSNs represent a huge black box that may hold the key to understanding the origins of HD pathology. The following pressing questions can be immediately answered with the right experiments.

- Is the primary defect presynaptic or postsynaptic? Striatal neurons become hyper-excitable in HD, but is this a primary or compensatory dysfunction?

- Are the changes cell-type specific, in D1 vs. D2 neurons?

- What is the role of voltage-gated calcium channels (VGCC) at these dysfunctional synapses? Examination of the calcium channels blockers can be undertaken immediately, using readily available genetic and pharmacological tools. Any positive results (with VGCC blockers) from other brain areas outside the BG might also be very useful to HD drug discovery.

- What changes occur in the homeostatic management of calcium in MSNs, particularly early in the pathology? Are these changes primary or adaptive?

Beyond the MSNs themselves, many other neuronal and structural components of the BG could hold critical clues to understanding HD pathology. Some of the questions surrounding these components include the following:

- What changes occur in the GABAergic interneurons of the striatum with HD? Recordings from this cell population in HD models could yield critical information.

- Are dysregulated inputs cortical, thalamic or both? GP inputs should also be considered.

- Are there primary or adaptive changes in other structures of the BG (including GP, STN, SNc, etc.)? We need to take a systematic look at how

HD affects the nuclei of BG. How are the nuclei related to each other, how are they related to cortical activity, and how is slow-wave activity affected? This would require recording from the output nuclei of BG.

- Are there changes in the autonomous activity of cells of GP, or in the synaptic properties of those neurons?

- Are there changes in the dopaminergic neurons of the SNc?

- As connectivity progressively decreases between MSNs and cortex (and possibly with other areas), might that be an attempt to restore their activity back to some target level? What signals trigger this process of disconnection? When MSNs lose those highly coordinated inputs—and thus their coordinated activation—what are the behavioral consequences for movement?

- After synapse and spine loss, what pushes neurons over the edge to cell death?

- If we could stop neurodegeneration today, could we restore striatal activity, and would that reverse the adaptation by those cells?

- We need to find ways to coordinate research efforts so that experiments in different systems can be standardized to some degree, and can thereby be done in parallel and compared with one another. This includes optimization of animal models. *In vivo* work must be done, but it must be informed by more reductionist slice work.

- In terms of building a computer model of HD, the problem is not that we lack people with expertise to build good models, the problem is that we are still missing key pieces of the wild-type BG circuitry. Once these are better understood, existing models of normal BG could be modified to accommodate HD.

Experiment alerts: The following section contains suggestions for specific experiments that were outlined at various points during workshop discussions.

- Record spontaneous EPSPs from cortical pyramidal neurons and spontaneous EPSCs from MSNs. If cortical cells are the primary site of pathology, the two readouts should be similarly elevated.

- Using two-photon microscopy and caged glutamate, probe the postsynaptic element of MSN synapses in isolation, thereby removing presynaptic cells from the equation. This will determine whether the primary sight of pathology is pre- or postsynaptic. If, using this paradigm, you do not see the

pathological changes in synaptic activity that are present in HD models, you can conclude that pathological changes must be presynaptic (i.e. cortical or thalamic).

- Use BAC array approach (wild-type and or HD models) to screen for genetic differences between D1 and D2 neurons; systematically identify potential sources of D2 neuron vulnerability.

- Deliver a BBB-penetrable A2A agonist that would work postsynaptically at D2 (indirect pathway) neurons and measure electrophysiological and behavioral effects.

- Look at synaptic electrophysiological and or behavioral effects of blocking voltage-gated calcium channels (VGCC) with dihydropyridines (L-channel antagonists) in animal models of HD. Also look for consequences in terms of dendritic spine loss. An antagonist specific to CaV1.3 would be useful. Explore connection of VGCCs to IP3 receptor.

- In order to distinguish whether the striatal MSNs that degenerate in HD receive input preferentially from cortex or from thalamus, stain for expression of vGluT1 or vGluT2 at synapses in HD model.

- Using one of the activation/ablation technologies (manipulation with capsaicin, diptheria toxin, or light), target specific neurons in either the direct or indirect pathway MSNs and look for motor behavioral effects in vivo. Compare these effects with behavior seen in animal HD models. This information could be used to target specific neuron types in terms of therapeutics.

- Design tasks in primates (possibly measuring saccadic eye movements) that might differentiate activation of direct vs. indirect pathway neurons.

Appendix: Experimental considerations

Animal models of HD:
The primary difference between animal models and human HD is that neurons do not die in animals. Despite the limitations of mouse and other models, they can provide valuable information. We need to keep in mind that we are asking the animal model to recapitulate 40 years of pathology in a few months. Animal lifetimes can be scaled to some degree. An inducible model would be valuable to separate developmental components.

Advantages and disadvantages of three main animal models in use:

1) R6/2: disadvantages: only a partial gene fragment, difficult to breed mice, polyglutamine repeat is unstable. Advantages: symptoms appear within 4-5 weeks, widely used as standard. Even as an inferior model, it can provide valuable information.
2) Full-length transgenic BAC and YAC: normal htt present, mutant htt introduced in addition. Mice breed well and repeat is stable. Entire htt gene from human, also several other hundred kD of human sequence included. Not sure of implications of this.
3) Knock-ins (4-5 available; most-used is C140): replaces normal htt with mutant htt. Complication: 17 AA's of glu are followed by a stretch of pseudo-polyproline. Knock-in is made with a human clone, but polyproline stretch differs in human and mouse. May end up with human, mouse or mixture of both sequences. Not yet known what issues that creates.

Stephani Sutherland 10/21/08 8:53 AM
Deleted:

Rat models: in order to make the leap to fully characterize a rat model, a compelling case must be made to change to rat. In the existing rat model of HD, motor symptoms arise in behavioral tests around 6 months; neuropathology is evident by 9-12 months.
Some attendees advocated for studies in rat (e.g., behavioral study advantages; last 10 yrs of *in vivo* recording has been done in rat, and new data could be directly correlated with that) while others prefer to stay with mouse (e.g. better characterization and genetic advantages, Cre lines can be manipulated). In the end, multiple models will probably provide the most robust information. Any experiments examining circuitry should be done in parallel with *in vivo* and slice work.

Slice preparations:

- Coronal: some cortical connections, but most connectivity to striatum is lost. Over 90% of synaptic plasticity studies use this prep.
- Horizontal: both cortical and thalamic connections remain intact.
- Parasagittal: cortical connections only, but more intact than in coronal slices.

Slice stimulation site:

- Within the striatum: advantage is that stimulus intensity can be lower; disadvantage is that you're activating all types of incoming fibers, not just glutamatergic fibers, which should be examined in isolation.
- In the cortex, using horizontal or parasagittal slices: requires higher stimulus intensity but allows for examination of fibers in isolation.

While multiple electrode array (MEA) recordings could potentially yield good information about cellular interactions and provide a good screening tool for compounds, the first step is to determine the basic synaptic dysfunction in single-neuron electrophysiology recordings.

Gap junctions: LV has shown in rat with double patch clamp that 1/3 of MSNs are electrically coupled. While there is no structural evidence for gap junctions, this type of evidence is very difficult to see. Using single-cell PCR, LV also found MSN expression of connexin 36 among other connexins.

Co-culture experiments can provide good insights in straight mechanistic studies, but they don't allow you to look at networks. A reason to use dissociated neurons is for good voltage control, but if you grow dendrites (the area of interest), there's no advantage of co-culture over using slices. The real advantage of the technique is to do genetic manipulations, look at the resulting activity, see transcriptional changes, morphological changes in the cells in isolation.

Pharmacological considerations:

- **A2A R antagonists** (and agonists) are heterogeneous, only some of which cross the BBB.
- A2A R antagonists may have differential affinity depending on heteromeric subunit composition.

- **Dihydropyridines** (L-type Ca channel antagonists) are heterogeneous, only some of which cross the BBB. Most commonly used drugs (for hypertension) do not enter the brain. These drugs work only at depolarized potentials, because they are allosteric regulators, not strict blockers or classic antagonists. They shift voltage dependence of activations. May only work when MSNs are in the upstate.
- An antagonist specific for CaV1.3 does not currently exist, but would be very useful. Small-molecule screening could yield one.

Reactive oxygen species (ROS): JS maintains that all estimates are artifactual.