

# Hereditary Disease Foundation

## Neural Progenitor Cells and Novel Regenerative Strategies for Neurodegenerative Diseases

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Prepared by Marc S. Hurlbert

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## ABSTRACT

Huntington's disease (HD) is a genetic neurodegenerative disorder that results in psychiatric symptoms as well as progressive movement abnormalities including chorea and excessive involuntary movements. As the disease progresses, the HD patient needs constant care and support, is unable to walk or swallow and, inevitably, dies prematurely. There is no cure for HD. It is the primary goal of the Hereditary Disease Foundation (HDF) to promote the discovery of a cure or treatment for HD. One primary strategy used by the HDF is to gather small groups of scientists to brainstorm and develop experiments that will lead to a greater understanding and possibly a cure for HD. The most recent workshop, *Neural Progenitor Cells and Novel Regenerative Strategies for Neurodegenerative Diseases*, covered the topic of utilizing stem cells for the treatment of HD. Experts on HD, stem cell research, CNS developmental biology, and neural transplantation attended the workshop. For two days, this diverse group of scientists discussed multiple strategies for implementing stem cells into HD research. The workshop concluded with a list of experiments to be tested in the laboratory. The main objective of this workshop was to design experiments to test stem cells as a potential therapy for HD.

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Dr. Allan Tobin, Scientific Director of HDF, opened the workshop by greeting the attendees. He established that the goal of the workshop was to strategize ways of implementing stem cells into the treatment of HD. Dr. Tobin stated that the HDF has been working with small groups of scientists for a number of years to come up with ideas and experiments within the HD research field to be tested in the lab and hopefully lead to a cure for HD. Each of the attendees introduced themselves and stated their area of expertise in scientific research. Dr. Tobin stated that the Foundation is not academic and is driven by the goal to cure HD. The Foundation was created in

1968 by Dr. Nancy Wexler's father after her mother had been diagnosed with HD.

The workshop began with the presentation of a videotape of patients with HD in the Venezuelan community. The video provided some background information on HD. It presented the clinical symptoms of HD to the basic research scientists, clearly showing how devastating it is to individuals and families. The HD patients shown on the videotape spanned a wide-range of HD phenotypes from late adult-onset to juveniles with onset at 6 years of age.

HD is caused by a CAG repeat that is expanded in the HD gene. This CAG repeat is translated into a stretch of glutamines in the affected protein, huntingtin (htt). Unaffected individuals have 15 to 34 CAG repeats in exon 1 of the HD gene. Individuals with 40 or more CAG repeats will develop HD. The age of onset of symptoms is inversely correlated with repeat length. Juvenile-onset HD patients, who usually have >60 CAG repeats in the HD gene, can have greater than 100 CAG repeats.

Dr. Anne Young made a comparison between adult-onset and juvenile-onset HD. She pointed out that juvenile patients are more rigid and stiff, slower, more dystonic and resemble Parkinson's disease patients rather than HD. Juvenile-onset patients usually have more severe cognitive decline, including loss of executive functions and psychiatric disturbances, particularly depression, manic depression and at times delusions and hallucinations, irritability, violence, and obsessive-compulsive symptoms. Adult-onset and juvenile onset patients tend to have excessive, uncontrolled movements. At the end-stage of HD, adult-onset patients may also become dystonic and then resemble the juvenile-onset HD patients. The neuropathology of HD, primarily degeneration of medium-spiny GABA neurons in the caudate and putamen, is more severe in juvenile-onset patients. In addition, other brain regions (cerebral cortex and basal ganglia) are mildly affected in adult-onset patients and show more pronounced degeneration in juvenile-onset HD patients. Dr. Young pointed out that there is no direct substantia nigra degeneration in the neuropathology of HD.

Very few brains of young juvenile-onset HD patients who died early in the course of the

Juvenile-onset HD is usually inherited from affected fathers due to very unstable CAG repeats in sperm. Data from HD patients in the United States show that, from age of onset, juvenile-onset HD patients live approximately 10 years, compared to adults who live about 20 to 25 years. Survival is not as good in other countries such as Venezuela. HD patients must intake five to ten times the daily calories to maintain body weight. Seizures are common in juvenile-onset HD patients, occurring in 16% of HD children, but they almost never occur in adults as a result of the disease. Seizures are sometimes, but not often, the cause of death in HD.

Many interesting phenomena have been observed in the Venezuelan HD families. The most severely affected juveniles usually come as the last offspring in a family. Another noteworthy point was made about repeat length. Although in the overall affected HD population the number of CAG repeats inversely correlates with age-at-onset and severity of symptoms, this does not always hold true. One HD woman from the Venezuelan group who had onset at age 14 had a daughter who developed HD symptoms at age 6. The daughter had 3 fewer CAG repeats in the HD gene than her mother. Another interesting case was a male with late onset at about 60 years of age. His wife also carried the HD mutation. Together they had 14 children and have almost 100 grand- and great-grandchildren. Some of their progeny carry expanded CAG repeats on both copies of the HD gene. To date, there does not appear to be a dosage effect as far as having two mutated copies of the HD gene. However, Dr. Wexler said there are too few examples of patients homozygous for the HD mutation to draw any conclusions.

Dr. Wexler and Dr. Young have been observing and helping this community for approximately 20 years. The Venezuelan population has more juvenile and young onset patients than the average. Dr. Wexler stated that research studies are underway to determine if this is because in the community they have longer repeats than other populations, or that other as yet undiscovered modifying factors, such as environment, sanitation and medical care, contribute to early onset of the disease.

Dr.

disease are available for study.

Ronald McKay asked if the Venezuelan families have frequent infectious diseases. Dr. Wexler replied that the Venezuelan community has a higher incidence of many disorders, including viral, bacterial and parasitic infection.

Many of the participants raised questions regarding the neuropathology of HD. What is the neuropathology of HD? Is striatal degeneration the only important neuropathology of HD? Dr. Young responded that we know the sequence of neuronal death in adult-onset HD. Primarily, degeneration occurs in the caudate and putamen of the striatum. Secondly, degeneration is observed in other regions including the cerebral cortex, hypothalamus, thalamus and components of the basal ganglia. The areas involved in adult-onset HD are more affected in juvenile-onset HD. Adult-onset has only subtle cortical involvement where juvenile-onset has very obvious cortical, pallidal, and thalamic degeneration. In juvenile-onset, not enough brains at varying stages of disease have been available to study the progression of degeneration.

Dr. Alex Joyner asked if a developmental defect could be ruled out. Dr. Young stated that they cannot disregard developmental defect but added that PET and MRI studies have not shown developmental abnormalities in young presymptomatic gene positive individuals.

In addressing Dr. Joyner's question, Dr. Mark Mehler stated he believed there was such a defect. He believes the affected individual's brain cells are developmentally set up to be more susceptible to environmental stress and therefore die more easily than cells with normal huntingtin protein (htt).

Dr. McKay asked a question about the appearance of HD in Venezuela versus the United States. Is the duration of HD similar? Dr. Wexler replied that the duration was longer in the U.S. because of better medical care.

## NEURAL PROGENITOR CELLS

Dr. Fred Gage introduced and compartmentalized four key points to consider when thinking of ways to implement stem cells into HD therapy.

1. Isolate cells (embryonic, postnatal, adult), expand in culture, and then pursue a specific lineage for neural transplantation. Concerns with this approach include phenotype commitment, migration, and synapse formation *in vivo*.
2. Stimulate the proliferation of endogenous multipotent cells with cytokines and growth factors. Are they similar to the embryonic cells that we can manipulate *in vitro*?
3. Use stem cells derived from HD patients to screen drugs in cell culture.
4. Pre-implantation diagnosis. How important is the status of clinical state before selecting what therapy to implement?

The overall goal of the workshop was to develop a means by which we could determine new useful information, innovative experiments and collaborations from the discussions. Dr. Paul Patterson stated that he realizes there is a dichotomy between those who prefer *ex vivo* manipulation of stem cells over *in vivo* expansion of the endogenous progenitors. He hoped that experiments could be designed during this workshop to test both paradigms.

Dr. Joyner asked if there was any hope that neural transplants could work? Could proper connections be regenerated? Dr. Gage answered that the initial results of human neural transplantation for Parkinson's disease (PD) looked promising. He added that there was a long history of fetal tissue transplants for HD in rat and primate lesion models. The transplants

Dr. Susan Hockfield brought up the topic of transplantation for HD and what was the best animal model to test stem cell neural transplants. Dr. Young stated that many HD mouse models

seemed to work in animal models of HD. Grafted fetal tissue survived and created a "striatal-like organism" growing in the brain. This new striatum can make proper synaptic projections and connections. He offered the idea that perhaps fetal striatal cell transplants are the wrong source of cells and a more appropriate stem cell would function even better.

Dr. Derek Van der Kooy was concerned about diffuse neurodegeneration in HD and asked how certain we were that replacing only striatal neurons would be sufficient to correct HD symptoms. He asked, "Are cortical transplants also necessary?" He believes *in vivo* expansion of endogenous progenitors will likely be more useful for the diffuse degeneration observed in HD.

Dr. Young stated that twenty years ago scientists would have said that the primary neuropathology of HD was 99% striatal degeneration. She suggested more degeneration must be occurring based on relative brain weights. The average human brain weighs 1400g, while the average adult HD brain weighs only 900g. The entire striatum only weighs 70g, and, therefore, more tissue must be degenerating throughout the HD brain.

Dr. Anthony Schapira commented that he has been pleasantly surprised at the positive results in neural transplantation for Parkinson's disease (PD). The clinical improvement reported to date was amazing. He agreed with Dr. Van der Kooy and had doubts that cell transplants would work as well in a more diffuse and advanced neurodegenerative disorder like HD. One important question he raised about HD was whether we would be transplanting cells into a toxic environment that would lead to the death of the transplanted cells. Could wildtype cells survive in the HD striatum?

were now available. Dr. Gage said two sets of transplants had to be carried out: First, to transplant wildtype cells into the HD mouse brain; and, second, to transplant HD mouse tissue into wildtype striatum. The first set of

transplants would address how transplants functioned in the HD brain. He emphasized researchers first needed to examine if there was neuroanatomical correction and, second, behavioral correction of the HD phenotype.

The second set of experiments would determine if the cells with mutated htt die in a cell autonomous fashion. Dr. Ethan Signer stated that such research could determine if HD resulted from the murder of sick cells by other cells in the brain or that it was suicide of the already sick cells.

Dr. Mehler agreed with the idea of activating endogenous progenitors. He stated that if there were presymptomatic markers, we might be better able to target our therapeutic approach.

Dr. Gage added to his remarks about the possibility of neural transplantation functioning as a therapy for HD. He stated that the initial fetal tissue transplants in HD patients were inconclusive. The patients were not systematically evaluated. No miracles had been reported to date. Controlled neural transplant studies in HD patients were needed.

Dr. David Anderson asked if there was an autoimmune component that triggered the neurodegeneration in HD. Dr. Young replied that years ago researchers examined that possibility and did not see any autoimmune component. However, she stated, that those studies were not thorough and given the fact that there is an inflammatory component to the degeneration in PD and Alzheimer's disease, further studies on inflammation in HD are needed. She added it is known that inflammatory cytokines are increased in the HD brain. Dr. Schapira added that the Bates HD mouse (R6/2 line) found markers of induced inflammation in the striatum, namely INOS and nitrotyrosine expression, and that there was some initial correlation to human HD. Dr. Anderson suggested crossing the HD mouse strain with an immune deficient background and then examining the resultant phenotype.

#### **DISCUSSION OF THE DIFFERENT HD MICE MODELS**

Next, the discussion shifted to which is the best mouse model to perform stem cell transplant

experiments. Many HD mouse models exist (see Table). Briefly, there are transgenic mice which express a truncated fragment of the mutant HD gene [*HD* exon 1 with ~150 CAG repeats, Prion:*HD* exons 1-3 with 82 CAG repeats, CMV:*HD* exons 1-3 with 89 CAG repeats, and *HD* ~1Kb of 5' with different numbers of repeats [unpublished report]. There are also transgenic mice which express the full-length human HD gene [CMV:*HD* full-length with 89 CAG repeats and 4p16.3 YAC with 72 CAG repeats]. Finally, there are mice with a targeted knock-in of the HD mutation (CAG repeats) into the mouse Huntington's disease homolog (*Hdh*) [MacDonald/Joyner *Hdh*<sup>109/+</sup>, *Hdh*<sup>90/+</sup>, *Hdh*<sup>n18/n109</sup>, *Hdh*<sup>n109/+</sup>, and *Hdh*<sup>n50/n50</sup>, Myers *Hdh*<sup>80/+</sup> and *Hdh*<sup>72/+</sup>, and Zeitlin *Hdh*<sup>94/+</sup> and *Hdh*<sup>71/+</sup> mice).

All of the above HD mouse models show some phenotype and will be useful in studying therapies and preventive interventions. The transgenic mice which express the truncated mutant *HD* gene are very useful for studying the inherent aggregation properties of mutant htt as well as for rapidly-progressing, early-onset movement disorders. The transgenic mice which express the full-length mutant *HD* gene show a later age-at-onset and remarkably are the only mice reported to show signs of significant amounts of neurodegeneration. Mice with targeted knock-in of CAG repeats into *Hdh* that express the mutant protein at levels similar to the endogenous mouse gene most accurately reproduce the genotype of human HD. The MacDonald/Joyner mice show a HD-like cellular phenotype which shows glutamine-length dependent and a dominant effect. Targeted knock-in mice from Myers and Zeitlin show aggressive behavior with reduced LTP in the hippocampus or glutamate sensitivity in striatal and cortical neurons, respectively. Finally, MacDonald/Joyner mice with targeted knock-in of CAG repeats into *Hdh* with reduced expression of mutant htt show both the HD-like cellular phenotype as well as a progressive movement disorder which resembles that of the transgenic R6/2 (*HD* exon 1 mice). In all, these various mouse models will prove useful for screening potential therapies and preventative strategies for treating Huntington's disease.

Interesting points to consider about the available mice include the developmental defects in the *Hdh<sup>neoQ50/neoQ50</sup>* mice of Joyner/MacDonald as pointed out by Dr. Van der Kooy. The mice have lowered protein levels (due to the *neo* cassette upstream of Exon 1) and there is a clear developmental defect including premature death and enlarged ventricles in the striatum at birth. Dr. Joyner stated the conditional knockout mice would be important in determining the role of lower htt protein expression on HD disease phenotype. Dr. Signer told us of an inducible mouse which has tetracycline-regulated promoter. Htt must be expressed during embryogenesis or else it results in embryonic lethality.

The question of which is the best HD mouse can be answered by a posing few questions: What do we want in a HD mouse model? Do we want a mouse with neuropathology like HD, or a mouse with behavioral abnormalities? Are both behavior and neuropathology necessary? Dr. McKay pointed out that one thing is clear in HD: there is massive degeneration in the striatum. This phenomenon cannot be overlooked. The primary cells that die are medium spiny GABA projection neurons. Our initial goal should be to get a stem cell to become a medium spiny GABA neuron *in vivo*. Dr. Gage agreed, and stated the initial goals should be to get survival of stem cells with the correct phenotype and neural projections and to show that the transplanted cells project to the globus pallidus and other targets. If a transplanted stem cell were GABAergic and projects to the globus pallidus, that would be a major step towards treating HD. Whether a stem cell transplant can then impact the clinical symptoms, namely to correct the abnormal behaviors, should then be examined.

Dr. Mehler agreed that using behavior as the endpoint marker for judging the success of a transplant is asking too much. He suggested we should look initially at whether the transplanted cells survive, differentiate into the correct neuronal phenotype, and behave like

There was some discussion over recently published data that will be useful in determining which cells to transplant in initial experiments. Dr. Anderson noted that Dr. Alvarez-Buylla's group and others have recently found the

endogenous striatal neurons as far as electrophysiology, biochemical characteristics, and receptor expression profiles. Dr. Luskin pointed out that we needed to define when to transplant cells. She asked, "Are we trying to deliver replacement cells or rescue dying cells?" Dr. Wexler urged that we begin transplants of a few cell types into a few of the available mice and examine the effect. Researchers could then look at the survival and projections made by the transplanted cells, and at the behavior in the transplanted animals. They could perform a few initial transplant experiments, learn from them, and then determine what approach would be best for the next round of transplants. Dr. Anderson agreed, but suggested that HDF could coordinate a few defined transplant experiments with some labs and set specific goals and possible outcomes for each experiment. He felt that a coordinated approach, would yield more information than a blind trial-and-error approach.

#### **EXOGENOUS STEM CELLS: WHICH CELLS TO USE? WHERE TO PUT THEM?**

Next, the discussion shifted to what cells are the best to transplant. There was a lot of debate over defining a *multipotential stem cell*. The participants defined the difference between cell fate and potential. *Cell fate* is what phenotype the cells may adopt *in vivo*. However if the cells were put into a different context in culture or transplanted to a different site in the brain they may become another type of neural cell. That is what defines a *stem cells potential*. It was agreed that all the currently available cells have the potential to differentiate into GABA neurons. It seems other neuronal phenotypes (glutamate, serotonin and dopamine) are not as easy to generate from multipotential stem cells. Given this information, it was suggested that all the cells including: fetal medial and lateral ganglionic eminence (MGE and LGE) cells, neurospheres from both adult and embryonic tissue, c-myc-immortalized cells, and the growth factor expanded cells be used in initial transplant studies.

progenitors derived from MGE migrate and integrate much more than LGE progenitors when transplanted either into the striatum or the cortex. Therefore, MGE cells are more attractive for use in initial neural transplant experiments. In addition, these initial experiments will show the

stem cell that most closely differentiates into a medium spiny GABA neuron can then be selected for other transplant studies. Markers to examine in the transplanted cells include the GABA synthesis enzymes (GAD67 and GAD65) and the dopamine receptors.

Dr. Gage was very interested which cells send out the correct projections and make synapses with the host brain cells. Dr. Snyder said his group could make the multipotent stem cells become GABAergic projection neurons but that he needed to know if that was sufficient. Dr. Gage said those transplants should be made and see if the transplanted cells can become striatal-like. That would be a significant first step. If we can show the transplanted cells survive and make correct projections, then experiments to test the correction of behavior could be tested.

## **ENDOGENOUS STEM CELLS**

*In Vivo* stimulation of endogenous stem cells was the next topic of discussion. Dr. Luskin reported that she observed the generation of new neurons in the adult rat striatum, after the infusion of BDNF into the lateral ventricle for 12 days. Using BrdU pulse labeling after the BDNF infusion, Dr. Luskin determined that 30% of the new cells were neurons. She believed this was due to enhanced proliferation of endogenous progenitors that then differentiate into neurons. Dr. Van der Kooy said he hypothesizes three events happening when BDNF is injected. First, there is increased survival of progenitor cells. Second, the BDNF could possibly stimulate proliferation of the stem cell pool. Lastly, BDNF must drive some progenitors to differentiate into neurons. The stimulation of the stem cell pool by BDNF is a possible explanation for the Luskin group's findings, but this needs to be confirmed. Dr. Goldman was surprised at Dr. Luskin's data. He had not initially observed as large of an effect as Luskin's group when he injected an adenovirus expressing BDNF into the ventricle. However, his group is still analyzing their results. Drs. Anderson and Van der Kooy stated that different doses of BDNF delivered (comparing viral vector delivery to direct infusion) could

Dr. Gage stated that many different progenitor cells should be transplanted into a few of the available HD mice in order to examine their

have led to the different findings between the two groups.

Dr. Alvarez-Buylla agreed with Steve Goldman's findings. BDNF infusion does not result in the generation of new neurons in his group of experiments. In addition, it is unclear what effects other growth factors, such as infusion of EGF or FGF, have on the striatum. Clearly, further confirmation of Dr. Luskin's findings of generation of new neurons in the striatum following BDNF infusion is necessary. Further characterization of the effects of infusion of other growth factors such as EGF and FGF into the striatum should also be carried out.

The first day of the workshop adjourned with a challenge issued by Dr. Gage to the participants not to be concerned with the details of the biological issues that need to be resolved, but rather to focus on possible therapeutic prospects. He posed the question, "What experiments could HDF fund today?"

## **PLANNING EXPERIMENTS**

Dr. Tobin opened the workshop by suggesting the participants should continue to talk about experiments that need to be done. He stated that we should consider transplantation not only for therapy but also to be used for classical developmental biology experiments. Dr. McKay said simple experiments could test out in the near future to show that stem cells could differentiate *in vivo* into the desired cell type. Progenitor cells should be transplanted into HD mice or lesioned animal striatum. After a few weeks, the animals could be harvested and the phenotype of the transplanted cells should be examined. He stated that within a month or two, initial data on stem cell transplants in HD would be available. Everyone emphasized that the question of whether the sick cells continue to die after transplantation into a normal striatum needs to be answered. Does the HD brain exert a toxic environment and transplanted cells die, or are only cells with the HD mutation vulnerable to degeneration?

engraftment, survival, projections, and connectivity. He recommended looking at histology first and behavior second. Dr. Snyder emphasized that stem cells have a greater

capacity than fetal tissue to differentiate into a variety of cell types post-transplantation. He added that stem cell transplants may better reconstruct the endogenous striatum including projection neurons, interneurons, and astrocytes.

Dr. Luskin agreed and stated that we may need to address which time point is most appropriate for transplantation: at birth, before clinical symptoms appear, or after clinical symptoms begin. She posed the question, "Which time point will be most applicable to treating human HD patients?"

Dr. Young stated that each of the HD mice available share some characteristics of human HD. It is difficult to classify one HD mouse as better than another. The Bates R6/2 mice have been the best characterized. However, the neuropathology, cell counts, receptor expression patterns, neurochemistry and electrophysiology need to be thoroughly studied in all of the HD mice. After that data has been gathered, we can better determine the desired effect of transplanted stem cells.

Dr. Gage suggested that Dr. Luskin's group should infuse BDNF into some of the HD mice and examine whether new striatal neurons are born. Do the new striatal neurons form intranuclear inclusions? Dr. Luskin asked about the infusion of other factors like EGF. Dr. Van der Kooy replied that EGF-infusion experiments showed proliferation and migration of endogenous precursors. He has observed that the EGF-stimulated precursors die after they have migrated away from the ventricle.

Dr. Anderson recapitulated a list of proposed experiments for the HD mice that he had heard over the last 2 days:

*1. Transplantation of fetal MGE or LGE into striatum;*

*2. Transplant naïve stem cells into the striatum;*

Dr. Alvarez-Buylla asked if exercise helps regenerate the brain. Dr. Gage has seen increased neurotrophin expression as well as a two-fold increase in neurogenesis in the dentate

*3. Transplant stem cells which have been partially guided down a neuronal path in vitro into the striatum; and,*

*4. Infuse growth factors into the ventricle of HD mice.*

Dr. Signer stated the Cure Huntington's Disease Initiative (CHDI), a part of HDF, was prepared to fund scientists working on experiments that have the potential to lead quickly to a cure or therapy for HD and to foster collaborations between experts.

Dr. Patterson said that one critical factor is the availability of the different HD mice. Dr. Signer assured the participants that the HDF and Jackson Laboratories were arranging for HD mice to be available to anyone who wants them for research. It was pointed out that the Bates R6/2 mice are currently available from Jackson Laboratories. Dr. Tobin stated that the HDF is assembling a reagent bank of the various antibodies, DNA constructs and other tools researchers need and for scientists to contact HDF about reagents as required.

In reviewing prospective experiments, Dr. Signer stated that transplants should be done to correct behavior in the mice that do not have apparent cell death, namely the R6/2 mice.

Dr. Young emphasized that other basic science experiments need to be performed on the HD mice. One approach her group has taken is to look at differential gene expression in the striatal tissue of HD mice as compared to controls. Her group has analyzed Affimatrix Gene Chip arrays and found a lot of changes in neurotransmitter receptors and growth factor expression. They have not observed huge changes in cell death molecules between the two groups.

gyrus following exercise. These effects have not been observed in other brain regions. However, a thorough examination of other brain regions needs to be carried out. It was suggested a similar study be performed on the HD mice. Will

exercise induce the generation of new neurons in the striatum?

Dr. Joyner asked, is it possible that there is a depletion of SVZ progenitors in the HD brain. Dr. Gage said the experiment has not been done, but that it could be performed by looking at BrdU incorporation after exercise, comparing control and HD mice. Dr. Van der Kooy stated that the number of sphere-forming cells does not change with either age or disease; rather, there is a decrease in later stage progenitors.

Dr. Young suggested making neurospheres; EGF or FGF2 propagated- progenitor cells from HD brain tissue. Dr. Vescovi volunteered to make the neurospheres because he has a wealth of experience making neurospheres from human embryonic tissue. Dr. Wexler stated she would help get the HD embryonic tissue to Dr. Vescovi.

What more do we need to learn in order to know what to do next?

Dr. Tobin started the final session by asking, "What is the next step to get experiments underway? Who wants to step forward with experiments to perform now and tomorrow?"

Dr. Schapira asked, "How toxic is the HD human striatum? How toxic is the HD mouse striatum?" He wondered about biochemical and receptor abnormalities. The R6/2 HD mice have receptor alterations as early as 4 weeks, nuclear inclusions of considerable size by 8 weeks, severe biochemical abnormalities at 12 weeks and death at 14 weeks. Transplants will need to be made prior to the earliest changes. He stated that the areas of interest would be to start transplants into the R6/2 mice with early symptoms -- GABAergic fetal tissue as well as the variety of stem cells available. He said we should look at whether transplants prevent the severe weight loss observed in the R6/2 mice. He suggested looking at biochemical changes before and after symptoms in the these mice. Maybe there will be

Dr. Alvarez-Buylla argued that the data on the proliferation of endogenous precursors stem cells by infusion of growth factors is inconclusive to date, and that further experiments to confirm whether growth-factor infusion leads to the

some marker or some way to track the state of the diseased brain.

Dr. Schapira has looked at defects in peripheral tissues. He found a defect in the Vmax of muscle tissue following exercise in HD patients, which correlates with CAG repeat length. The same is true with other presymptomatic patients with HD. The Vmax defect is prior to onset of clinical HD. That is a big finding. This could be a pre-symptomatic test to track disease progression in HD patients. Dr. Gage asked how big the effect was. Dr. Schapira stated that the drop in Vmax in HD from normal controls is ~40%. He said the controls had a significantly higher Vmax than presymptomatic individuals, but that presymptomatic persons performed significantly better than symptomatic patients with HD had.

Dr. Tobin asked if the biochemical measurement using 2-deoxyglucose (2-DG, a measure of brain metabolism) had been performed in HD mice. Dr. Young stated that Susan Brown has been performing these studies but no results have been reported yet.

It was suggested by many of the scientists that Dr. Schapira track the similar muscle biochemical studies in the Bates R6/2 mouse. Dr. Flint Beal has seen similar defect at rest in HD patients. Dr. Schapira found the defect was more pronounced after exercise.

In closing, What experiment would you most likely do?

Dr. Tobin asked each investigator, "What is your favorite experiment to do?"

Dr. Van der Kooy would do one experiment: the infusion of growth factor (BDNF, TGF) into the ventricle of HD mice. He would track new neurons in the striatum and examine inclusion formation in those new cells.

generation of new neurons should be carried out in normal mice. He would prefer to see transplants of fetal MGE as well as cortical tissue into the HD mice. He would also pursue understanding the developmental origin of the medium spiny GABA neurons of the striatum.

Dr. McKay would use primary cells to show that the pool of primary fetal medium spiny neurons could be expanded in culture. He would then graft these expanded medium spiny GABA neurons into the HD mice. He would examine morphology and survival as well as behavioral recovery in the mice who received transplants.

Dr. Wexler believes it is imperative to have markers of disease progression prior to the onset of clinical symptoms. She was impressed by the finding of a decreased Vmax in muscle tissue. This defect, combined with gene testing and brain imaging, are avenues she would pursue to answer the important questions: When is the best time to intervene with a stem cell transplant? What intervention is appropriate: fetal tissue, stem cell, and/or drug therapy?

Dr. Joyner stated that she had enjoyed learning more about HD. In collaboration with Marcy MacDonald, she has generated many HD knockout and transgenic mice. Dr. Joyner believes a cell therapy may not be useful for HD and that we should probably focus on ways to keep the mutant htt from aggregating in the nucleus. She felt it is imperative to study the different HD mice and fully characterize their “progressive” phenotype (biochemically, neurophysiologically, and behaviorally). She proposed that HD might be a loss-of-function mutation resulting from loss of functional htt. This loss of functional htt leads to neuronal dysfunction and eventually cell death. In terms of mouse models, we need to examine the current mice who express different forms and levels of the htt protein. The mice who express different levels of full-length htt (either overexpression, equal expression, or reduced expression) should be compared. The conditional knockout mice might give valuable information regarding whether loss-of-functional htt in the adult striatum leads to HD-like symptoms.

Dr. Anderson believes the loss of function and gain of function of mutant htt could yield different neuropathology. This can only be studied in mice expressing different levels of

Dr. Snyder would employ both the therapeutic as well as the basic science approach to study HD. He liked the idea of cellular interventions. He would transplant wildtype stem cells into the embryonic HD mouse *in utero* and determine

wildtype and mutant htt. He would most like to see fetal MGE and LGE transplants in the HD mice. Anderson would track presymptomatic markers as a measure of whether the therapies are working.

Dr. Stern would use neural transplantation and stem cell in the HD mice to study neuropathology and gain a better understanding of HD as a way to reveal basic biology (not as a therapy). He believes experiments need to be done to yield more knowledge about the disease before searching for a cure. The most important questions he would try to answer are: How many different pathologies combine to make the disease state of HD? Is HD a gain-of-function or a loss-of-function disease? Is only the brain involved? What about other tissues like muscle and pancreas? And, finally, what is the normal function of htt protein? He emphasized that transplants need to be perfected in HD mice prior to any experiments in humans.

Dr. Luskin would exploit presymptomatic markers in mice and man to influence the therapeutic approach. She agrees the infusion of BDNF to stimulate endogenous precursor proliferation needs to be carried out in the HD mice. She also stated that it is important to classify the similarities and differences between the various HD mice and human HD.

Dr. Goldman disagreed with therapy driven studies. He preferred to ask more basic questions, like what happens when mutant cells are transplanted into normal tissue? Or, the converse, what happens to normal cells when transplanted into mutant tissue (HD mouse striatum)? He would utilize neural transplantation as a tool to study the biology and time course of neural damage in HD mice.

whether those wild type cells affect the phenotype of the mice. Could those transplanted cells be sufficient to rescue the mice from the disease? He would measure many things including neuronal phenotype, glial to neuron ratios in the striatum, neuropathology of the

striatum and cortex. Lastly, he would perform a biochemical analysis of the transplanted cells.

Dr. Vescovi would perform a systematic test of neural transplantation of the different stem cell types and examine their ability to influence the phenotype in the various HD mice. In addition, he agreed that growth factor propagated HD “neurospheres” derived from HD fetal tissue would be an invaluable tool.

Dr. Young believed generating cell lines from HD embryos was the best idea. She also liked the experiment of transplanting mutant cells into a wildtype striatum and determining what effect a normal environment would have on the mutant cells.

Dr. Patterson would transplant both committed progenitors as well as multipotent stem cells into the striatum and cortex of the HD mice. He would ask whether the cells survive and incorporate into the HD brain. Also, he would study the neural phenotype the transplanted cells adopt. He would also like to see BDNF infused into the ventricle of the HD mice and then study the new neurons generated in the striatum by such a treatment.

Dr. Signer agreed with Dr. Anderson that therapy-based approaches were important to determine what will work. He would like to see neural transplantation used both as a therapy and a tool in all of the available HD mouse models.

Dr. Hurlbert would like to see stem cell and neurosphere transplants into the available HD mice. He would look at the effect on animal behavior as well as examine the morphology, phenotype, projections and synaptic connections of the transplanted cells. He is prepared to carry out transplants in the Joyner/MacDonald mice that express lower levels of htt with 18 and 109 CAG repeats.

Dr. Mehler agreed with Dr. Stern’s cautious approach to studying HD. He would not jump into experimental therapies with humans until

5. Determine if the HD brain is a toxic environment. Transplant wildtype cells into HD mouse striatum and examine phenotype of cells a

few weeks later. Conversely, transplant HD stem cells (or fetal tissue) into normal mouse striatum and examine phenotype a few weeks later.

Dr. Gage would try to rescue mouse cells with an adeno-associated-viral vector expressing wildtype htt. He would inject into the HD mouse striatum. Gage would also like to see, if a stem cell transplanted into the striatum can become a mature, medium spiny GABA neuron with appropriate biochemical properties and synaptic connections.

## SUMMARY

In summary, over the course of this workshop the participants generated a list of experiments that need to be performed. Experiments range from basic experiments that will provide/be designed for a better understanding of HD to neural transplant experiments geared more towards discovering a therapy for HD. The suggested experiments follow:

### Basic Experiments:

1. Create progenitor cell-lines that carry the HD mutation. EGF or FGF2 propagated neurospheres will be generated from HD fetal tissue.
2. Characterize normal htt protein function. Examine mice which express different forms of htt and the conditional knock-out mice to determine if HD results from a loss of functional htt in the cytoplasm.
3. Characterize neuropathology in all of the HD mice models. Examine receptor expression, neurodegeneration, and biochemical abnormalities.
4. Determine if the muscle defect observed in human HD is present in the different HD mice models.

few weeks later. Conversely, transplant HD stem cells (or fetal tissue) into normal mouse striatum and examine phenotype a few weeks later.

6. Determine if medium spiny GABA neuron precursors can be expanded in culture from fetal striatal tissue.

#### **Neural Transplant experiments:**

1. Classical neural transplant experiment.

Transplant fetal MGE or LGE into HD mouse striatum and examine cell survival, projections, and mouse behavior.

-MGE transplants should be tried initially, as transplanted MGE cells migrate and integrate into the striatum and cortex (unlike LGE cells)

2. Transplant stem cells into HD mouse and lesioned-rodent models of HD.

-Determine which stem cells differentiate into the correct cell fate *in vivo*. (Namely, which cells can become medium spiny GABA neurons). Examine cell phenotype, projections, and connectivity after transplantation.

-Examine biochemical effects of a transplant (2-deoxyglucose recovery).

-Examine the effect a stem cell transplant has on clinical symptoms in HD mice.

3. Transplantation of genetically manipulated stem cells (which express “cell-death” genes) should be carried out in order to determine the effect on behavior and neuroanatomy of transplanted cells. Progenitor cells which express “cell-death” genes can be transplanted into HD-like mouse. The transplanted HD mice can be divided into groups to examine the effect of transplant: untransplanted HD mice, transplanted HD mice, and transplanted/cell-killed HD mice, in which the transplanted cells have been killed by treatment with antibiotic.

4. *In utero* transplantation of stem cells into HD mice embryos to prevent HD.

-Can the wildtype stem cells incorporate and function in place of the endogenous striatal cells which express mutant htt?

2. Follow up to experiment #1: If BDNF or EGF or FGF induce new neurons in the wildtype mouse striatum, infuse the appropriate growth factor into the ventricle of HD mice and examine if the new neurons generated in the striatum form nuclear inclusions of htt and whether they degenerate.

3. Rescue the cells with mutant htt in the HD mouse striatum by overexpressing wildtype htt. Deliver wildtype htt with an adeno-associated virus expressing htt.

4. Examine the effect of exercise on striatal neurons in wildtype mice. Follow-up these studies in HD mice if any effect of exercise (ie, neurogenesis) is observed in the striatum.

#### **Regenerative experiments:**

1. Infuse BDNF, EGF, and FGF separately into the ventricle of wildtype mice and examine if new neurons generated in the striatum. This experiment is necessary to confirm the Luskin groups' findings.

**TABLE 1**

***Expression of Huntington's Disease Mutation in Mice***

**Model design**

**Behavioral disorder**

**Neuropathology**

LAB	Background	Promoter/ gene size/ CAG repeats	Protein expression level	Clasping Phenotype	Rotarod	Other symptoms	NII Cell Pathology	Cell Loss	Brain Atrophy
<b>Transgenic HD models</b>									
Bates <sub>1</sub>	CBA/ C57B16	HD promoter exon 1 R6/2 144Q (expanded to 170 - 190 Q), R6/1 115 Q	RNA levels < 1x	Onset 2 mo. (R6/1 onset 5 mo.)	Abnormal by 5 weeks	Tremors & abnormal gait, learning deficit, hypokinesia, diabetes	NII and neuropil aggregates throughout brain, fewer dendritic spines, depol. Str. Cells	Frontal cortex, dorsal striatum and cerebellar Purkinje cells at late stage	Overall brain atrophy
Hayden <sub>10</sub>	FVB/N	HD YAC Full-length 72Q, 46Q, 18Q	2X	72Q (#2498) onset by 3 mo.	not reported	Hyperactive & circling	Inclusions in striatum	Cell loss in striatum	not reported
Ross/Borchelt <sub>2</sub>	C3H/B16	PrP prom. N171 82Q, 44Q, 18Q	1/5X to 1/10X	82Q onset 5 mo.	Abnormal by 3 mo.	Tremors & abnormal gait, hypokinesia, weight loss, early death	Inclusions: striatum, ctx, hippocampus, amygdala, cb. Diffuse nuclear accumu. of htt protein	toluidine blue reveals cells w/a degenerative morphology in the lateral striatum	Overall brain atrophy
Tagle <sub>3, 4, 5</sub>	FVB/N	CMV prom. Full-length 89Q, 48Q, 16Q	5X	89Q&48Q onset 4 mo.	not reported	Circling, hyperactivity, end-stage hypoactivity & urinary incontinence	Fewer inclusions throughout brain	20% cell loss in striatum of some animals	not reported
	FVB/N	CMV prom Ex1-3, 89Q	Endogenous to 2x	ex1-3:89Q onset 4 mon.	not measured	Prolonged Hyperactivity	Fewer inclusions throughout the brain	not reported	not reported
Aronin/ DiFiglia <sub>6</sub>	SJL/ C57B16	rat NSE 3 kb fragment 100Q, 48Q, 18Q	>endogenous about 1/5X	100Q onset 3-4 mo.	Abnormal about 4 mo.	Hyperactivity to endstage hypoactivity	Inclusions in Q100 (few in Q46), DN's observed	20% cell loss ~ 8mo. in some animals	Brain atrophy in some animals
Hen/Yamamoto <sub>7</sub>	CBA/ C57B16	tet-off (CamKII $\alpha$ -tTA) TetO Exon1 94Q	>endogenous	94Q onset in 50% by 2.5 mo.	Onset about 2.5 mo.	Late onset tremor & abnormal gait	Inclusions in striatum, septum, ctx, hippo., & reactive astrocytes	not reported	Brain atrophy & progressive striatal atrophy
<b>Targeted <i>Hdh</i> models</b>									
MacDonald/ Joyner <sub>11, 12, 13</sub>	129/CD1	<i>Hdh</i> promoter Knock-in 111Q, 92Q, 50Q	endogenous	no movement Sx.	no difficulties found	cellular phenotype see neuropath	CAG- & age- depend.HTT nuclear relocalization at 1.5 mo, inclusion >6 mo.	none observed	not reported
	SW	<i>neo</i> & <i>Hdh</i> prom., Knock-in 111Q,20Q	<50% of endog.	<i>Hdh</i> <sup>nQ20/nQ111</sup> have onset ~ 2 mo.	not measured	nQ20/nQ111 show disorders similar to Exon1-Q144 mouse	age- dependent HTT nuclear re-localization and inclusions	none observed	preliminary result: brain atrophy
Myers <sub>14</sub>	FVB- N/B16	<i>Hdh</i> prom., Knock-in 80Q,72Q	2X	no movement Sx.	none reported	Early onset aggressive behavior	Late inclusions, hipp. LTP impaired, repeat instability in striatum	none observed	not reported
Zeitlin <sub>15</sub>	C57B16	<i>Hdh</i> prom., Knock-in 94Q, 71Q	~2X	no movement Sx.	none reported	No movement Sx.; NMDA sensitivity	No inclusions	Striatal cell swelling in response to NMDA	smaller striatal cells
<b>Targeted Non-<i>Hdh</i> polyQ</b>									
Detloff <sub>8</sub>	129/ C57B16	x-link <i>Hprt</i> locus 146Q insert	endogenous	Onset ~5 mo.	none reported	Handling-induced seizures & early death	Inclusions throughout brain	None	not reported

## Transgenic DRPLA model

Ross/Borchelt <sub>9</sub>	C3H/B16	PrP Promotor, full length atrophin-1 cDNA(1184aa), 65Q and 26Q	about 1-3x (two lines)	less than HD	abnormal by two months	tremors, ataxia, twitchy movements, loss of coordination, seizures, early death	Widespread Inclusions, also accumulation of truncated N-terminal fragment of atrophin, similar to a fragment seen in DRPLA brains	approx. 30% loss in cerebellar Dentate nucleus(site of DRPLA path)	Overall brain atrophy
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Table Created by Marina Chicurel, Ph.D., Marc S. Hurlbert, Ph.D., Erik Schweitzer, and Ai Yamamoto March 2000

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