

Neurodegenerative Diseases: New Directions

The Gladstone Connection

By any measure, neurodegenerative diseases represent a rapidly growing medical and social problem. These diseases rob people of their ability to remember, speak, write, ambulate, and control their lives. There is no cure for these diseases.

Each year more than 50,000 Americans die with Alzheimer's disease (AD). In the United States alone, the number of AD patients is expected to more than double to over 12 million by 2050. More than 30,000 are afflicted with Huntington's disease (HD), and nearly 16,000 die with Parkinson's disease each year.

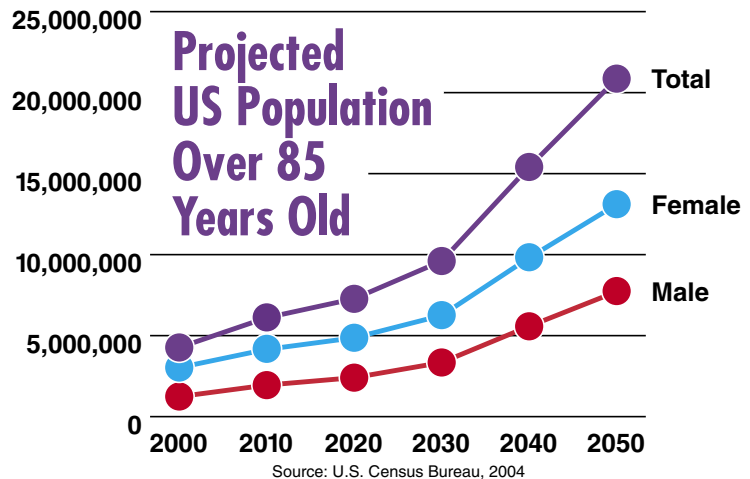
Despite these grim statistics, basic research into neurodegenerative diseases has accelerated in the past few years. Scientists at the Gladstone Institute of Neurological Disease have been at the very forefront of this rapidly advancing field.

The Gladstone investigators have focused on a common feature of several neurodegenerative diseases, including AD, HD, Parkinson's disease, and Lewy body disease. They hypothesize that, although different proteins accumulate in different neurodegenerative disorders, the ways in which they damage brain cells overlap. It may therefore be possible to develop treatments that help in more than one of these conditions. Most of the accumulating proteins form deposits (e.g., amyloid plaques in AD). However, it is not clear whether the deposits actually cause neurological deficits, are innocent bystanders, or represent beneficial defense responses. The scientists hope to resolve these and other questions.

Building a Better Mousetrap

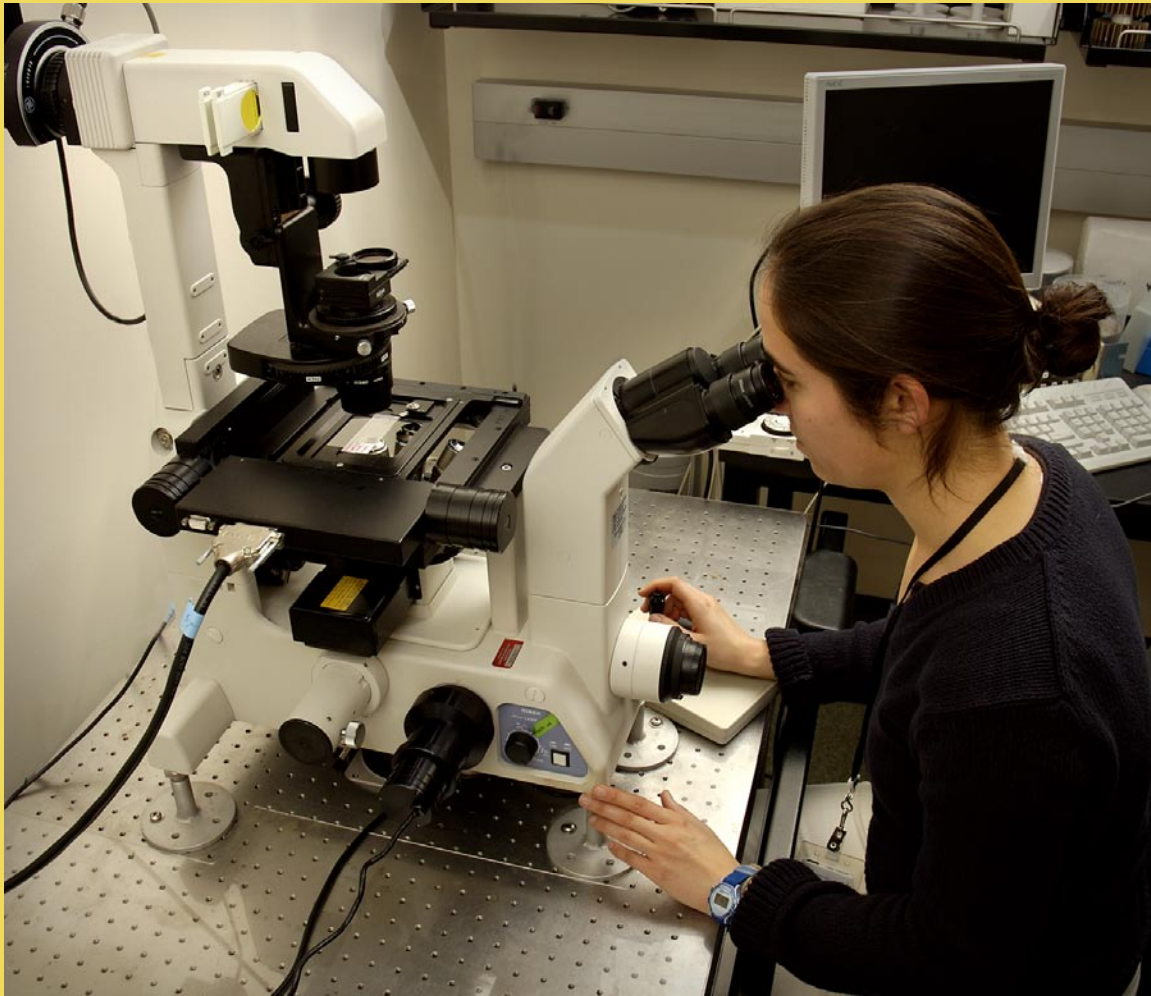
HD is caused by mutant forms of a protein called huntingtin (htt) that kill specific neurons. Within these cells, the mutant htt often aggregates into abnormal deposits, called inclusion bodies (IBs), which are found at the major sites of neurodegeneration—medium spiny neurons in a brain region called the striatum, which is critical for movement and other functions. For years, scientists have wondered if the IBs were causative, protective, or simply incidental.

Because conventional approaches had not resolved this impasse, Gladstone investigator Steven



Proteopathies of the Aging Central Nervous System

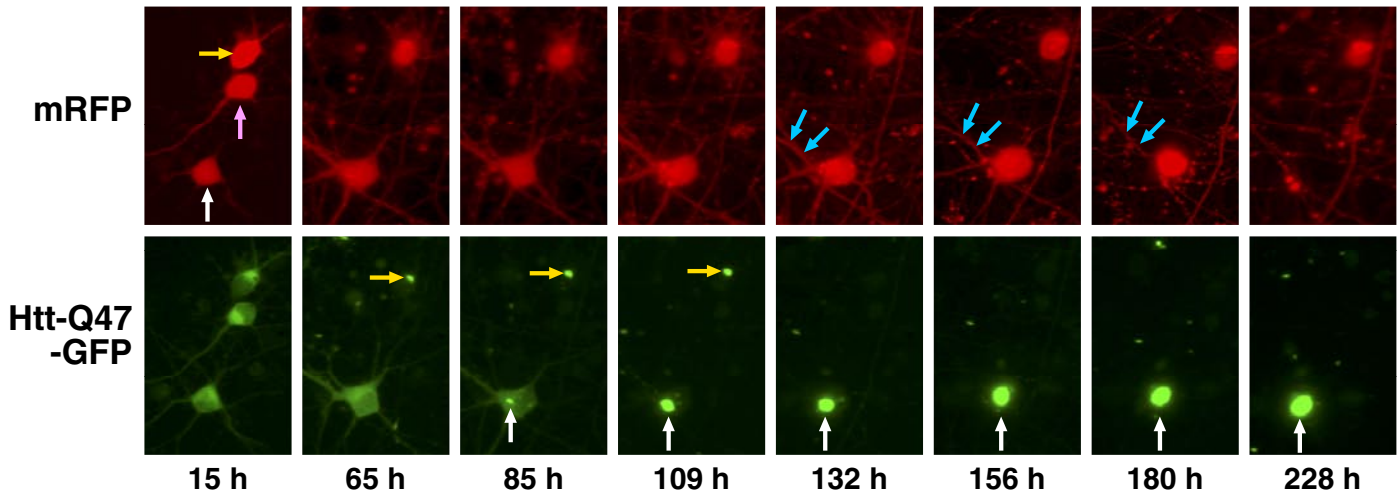
DISEASE	AFFECTED PROTEIN
Alzheimer's disease	A β , apoE, tau
Huntington's disease	Huntingtin
Parkinson's/Lewy body diseases	α -Synuclein
Prion diseases	Prions
Amyotrophic lateral sclerosis	Superoxide dismutase 1



THE ROBOTIC MICROSCOPE

The automated microscope consists of a standard microscope body with computer-controlled motorized stage and focus controls. The microscope can focus itself automatically by using a mathematical strategy called fast Fourier transform to calculate the sharpness of an image. The computer tells the microscope to move the focus knob back and forth, rapidly testing the sharpness of each image, until it finds the best one. Then the computer takes pictures of the neurons in the field, using different colors of light to reveal different types of information. Once the microscope has finished imaging one field, it moves the entire plate of cells to precisely position it on an adjacent field, and the whole process begins again. In minutes, it can finish the entire plate, imaging millions of neurons. After imaging, the neurons can be removed to an incubator until they need to be imaged again. Using a special strategy, the microscope can return to precisely the same neuron or field of neurons on another day.

Longitudinal analysis of single cells provides unique opportunities for elucidating cause-and-effect mechanisms. Because the automated microscope makes it possible to follow individual neurons throughout their lifespan, special statistical techniques can be used to provide information that cannot be obtained by conventional means. One of these techniques is called survival analysis. It can tell us how likely it is that a neuron will die and can measure risk factors for degeneration in individual neurons. This is important because previously we could observe factors but we had no way to relate them to that neuron's fate. Survival analysis tells us whether there is a relationship at all as well as the nature and strength of the relationship. With this approach, we can determine if a particular factor is likely to be incidental, beneficial, or pathogenic and whether it makes a major or minor contribution to determining a neuron's fate. Answers to these questions are essential for identifying the best therapeutic targets and for assessing the risk/benefit profiles of new drugs.



Finkbeiner developed a robotic microscope to collect images of individual cells as they change over time. By introducing fluorescently tagged versions of normal htt or disease-causing htt into neurons, he and his colleagues were able to use the robotic microscope to monitor IB formation, intracellular htt levels, and survival of individual cells. Sophisticated statistical techniques for survival analysis were then used to determine if a particular abnormality predicts early death and might be pathogenic or predicts longer survival and might be a beneficial coping response.

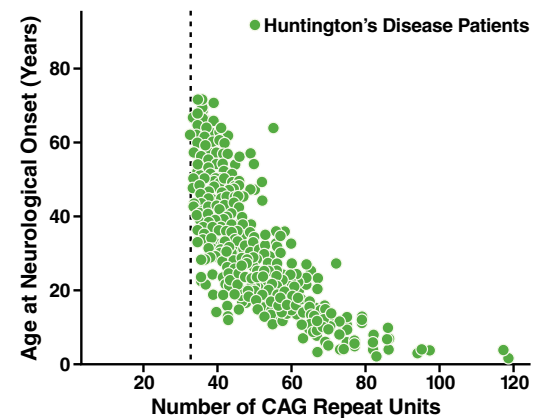
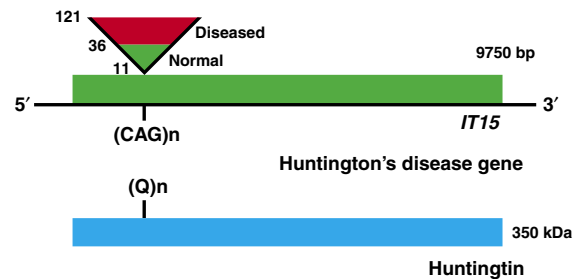
The risk of death was low in neurons transfected with the normal form of htt and high in cells expressing disease-causing htt. Furthermore, the risk of death increased with increasing size of the mutant polyglutamine stretch. These two findings were expected. However, the researchers also found that IBs formed by mutant htt reduced the risk of death in cultured neurons. Many scientists had assumed that IBs caused neurodegeneration. The robotic microscope was critical for uncovering this unexpected relationship.

So if the IBs protect neurons, what causes HD? One possible explanation is that the real culprit is a more soluble form of htt. When IBs form, the amount of toxic htt in the rest of the neuron is reduced, and the neuron survives.

Polyglutamine Diseases

Dr. Finkbeiner's findings may have even broader implications. HD is one of several neurodegenerative diseases characterized by accumulations of specific cellular proteins. Similar deposits, although of differ-

Polyglutamine-expansion-dependent cell death measured with an automated microscope by longitudinal tracking of single neurons expressing red fluorescent protein (mRFP, top panels) and a version of mutant htt tagged with green fluorescent protein (Htt-Q47-GFP, bottom panels). Two neurons (yellow and white arrows, top row) that formed IBs (yellow and white arrows, bottom row) outlived a third neuron, which died without an IB (pink arrow). Soon after an IB formed (white arrow, bottom panel), mutant htt disappeared elsewhere in the neuron. Neuron morphology remained intact for days (top row), but then neurites degenerated (blue arrows) and the neuron died. Thus, IB formation provided a measure of protection.



HD is caused by a polyglutamine expansion in the protein huntingtin. Huntingtin is a normal cellular protein, but when the number of polyglutamines surpasses 35, HD results. Similar polyglutamine expansions in different proteins cause at least nine other neurological diseases.

ent proteins, are also found in the brains of patients with AD, prion diseases, amyotrophic lateral sclerosis (Lou Gehrig's disease), Parkinson's disease, and a group of nine so-called polyglutamine diseases, of which HD is the most widely known.

The approach developed by the Finkbeiner group—combining the use of a robotic microscope with powerful techniques of statistical analysis—will undoubtedly be useful in studies of these diseases.

Proteopathies of the Aging Brain

The results from Dr. Finkbeiner's laboratory fit into the larger collaborative effort within the Gladstone Institute of Neurological Disease in that several investigators study how the accumulation of abnormally folded proteins causes neurodegenerative diseases.

The pathological hallmarks of AD are two types of protein aggregation: neurofibrillary tangles and plaques of amyloid β ($A\beta$). Lennart Mucke's research examines the molecular cascades that link accumulation of $A\beta$ to neurological dysfunction. Like Dr. Finkbeiner, he has found several disease markers that predict the loss of neurological function better than plaque accumulation. His previous studies demonstrated that the disruption of memory circuits in the brains of an AD mouse model correlate with $A\beta$ levels but not with $A\beta$ deposits, suggesting a plaque-independent role for nonfibrillar $A\beta$ species in the pathogenesis of AD.

The major known genetic risk factor for AD is the apolipoprotein (apo) E isoform apoE4. Karl Weisgraber is examining the structural differences between apoE4 and the more protective isoform apoE3, looking for associations between the unique structural features of apoE4 and either neurodegeneration or deficits in neuronal repair. Dr. Weisgraber has shown that, unlike the other apoE isoforms, apoE4 forms a stable folding intermediate (or molten globule) and displays an interaction between the amino- and carboxyl-terminal domains, causing the protein to adopt a more compact structure. His studies may pinpoint the biophysical properties of apoE4 that can be targeted therapeutically to block or diminish its pathogenic effects on AD and other neurological conditions.

Yadong Huang and Robert W. Mahley also study the role of apoE4 in the pathogenesis of AD. They have found that apoE4 undergoes intracellular cleavage at a greater rate than apoE3 and that apoE4 induces the phosphorylation of tau and the formation of structures resembling the neurofibrillary tangles seen in AD.

The multidisciplinary approach at Gladstone utilizes robotic microscopy, transgenic and gene-targeted mouse models, cellular biology, X-ray crystallography, quantitative neuropathology, and behavioral neuroscience to bear on a wide range of devastating neurodegenerative disorders. More importantly, Gladstone scientists focus on a particular aspect of these diseases, the critical injuries inflicted

by stealthy and particularly toxic forms of misfolded proteins that have not yet been sequestered in larger deposits. The results of their work have already changed the way neuroscientists think about these diseases and have contributed to novel strategies for the development of therapies.

Gladstone Research into Proteopathies and Neurodegenerative Diseases

Amyloid β in Alzheimer's disease	Lennart Mucke, Li Gan
α-Synuclein in Parkinson's disease	Lennart Mucke, Steven Finkbeiner
Proteolytic cleavage of apoE4	Robert Mahley, Yadong Huang
Structural examination of apoE	Karl Weisgraber
Inclusion bodies in Huntington's disease	Steven Finkbeiner