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Neurodegenerative Diseases

Where Are We Not Looking for Answers?

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Abstract

Preventing age-dependent neurodegeneration is a compelling yet elusive goal for neuroscientists, clinicians, affected individuals and their caregivers. As life expectancy increases and the global population ages, the burden of illness soars. A critical examination of what scientific areas require a fresh look is motivated by the growing magnitude of the health problem as well as by the numerous failed trials of agents once considered worth the investment of clinical trial resources in Alzheimer, Parkinson, and Huntington diseases. In so many of these cases it has become clear that the disease is more complicated than previously thought and that fatal flaws stemmed from assumptions based on imperfect or incomplete information from animal models. Renewed optimism that science will be able to provide relief derives from new knowledge that comes on a regular basis from bright, dedicated basic scientists. Lessons have been learned from failed experiments in the laboratory and clinic, and new tools are now available which allow questions to be addressed that were previously out of our reach.

By casting a wide net that tries to envelop the entire scope of the problem, this chapter considers the scientific gaps that stand in the way of effective treatments for neurodegeneration. Discoveries that present tantalizing therapeutic hypotheses are viewed as pieces of a puzzle so that the "magic" is stripped from the search for a "magic pill." At its core, neurodegeneration is a problem of cell health. Importantly, cells are interrelated within the brain, and thus limiting study to one type of cell is a flawed strategy. Neurodegenerative diseases must be considered as disorders of nervous system circuits. Patients' symptoms are the manifestations of neural circuit dysfunction. Accordingly, a successful treatment needs (a) to normalize the biology within individual cells as well as within the tissue and (b) to preserve or repair the information processing in important neural circuits. This approach places new biologic discoveries in perspective and promotes discussion of gaps that stand between discoveries and knowledge of neurodegeneration in overall tissues and circuit dysfunction that eventuates clinically.

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Introduction

Neurodegenerative diseases represent a major public health challenge that threatens to reach crisis proportions with aging of the population. The science of neurodegenerative disorders has exploded over the last decades, yet treatments for patients have not been forthcoming. A number of clinical trials of potentially disease-modifying drugs have failed in Phase III. Critical reappraisal of these therapy-development efforts yielded ideas on the major hurdles that need to be overcome as well as how future research could be improved (Huang and Mucke 2012). At this Ernst Strüngmann Forum we asked, "Where are the major gaps in our understanding and capabilities?" and "Where are we not looking for answers?" A number of recent brainstorming efforts provided background for this forward-looking exercise. The National Institutes of Health convened three workshops: the Alzheimer's Disease Research Summit 2012,[1] a workshop on Alzheimer's Disease-Related Dementias: Research Challenges and Opportunities,[2] and a conference on research planning for Parkinson disease in 2014.[3] In addition the Alzheimer's Disease Summit: The Path to 2025,[4] convened in 2013 by the New York Academy of Sciences, addressed major public health issues and posited strategic recommendations for research and the Institute of Medicine Neuroscience Forum held a workshop in 2012 on cross-cutting themes in neurodegenerative disease research (for a summary, see Institute of Medicine 2013).

A number of common themes have come to the forefront as scientists, physicians, patients, caregivers, governments, and private organizations take on the growing public health crisis of age-related neurodegeneration. Two general classes encompass the major issues:

1. There are significant gaps in our knowledge of the basic biology that underlie aging and neurodegeneration. Although scientists have identified a number of important key features of human neurodegeneration, we are far from having a full picture and forced to rely on tremendous assumptions. Leading therapeutic hypotheses have, out of necessity, been based on assumptions rather than on solid information. It is thus critical to fill major scientific gaps in basic understandings of neurodegenerative disease if rational treatments are to be developed.
2. The nature of the public health problem and the personal suffering of affected individuals and their caregivers demand that we continue

[1] http://www.nia.nih.gov/newsroom/announcements/2012/05/alzheimers-disease-research-summit-offers-research-recommendations (accessed April 9, 2015)

[2] http://www.ninds.nih.gov/funding/areas/neurodegeneration/workshops/adrd2013/ (accessed April 9, 2015)

[3] http://www.ninds.nih.gov/research/parkinsonsweb/PD2014/index.htm (accessed April 9, 2015)

[4] http://www.nyas.org/pathto2025 (accessed April 9, 2015)

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> to test the most promising therapies, despite incomplete knowledge. Past failures call for smarter approaches if we are to gain significant knowledge, even from negative trials. It is essential, for example, to know that a drug candidate engages its target in the human brain and ideally that it modifies the relevant biological pathway at therapeutic doses prior to a long and expensive Phase III efficacy trial. Assuring target engagement and pathway modification requires the development of new biomarkers based on the pathophysiology that occurs within the specific subgroup of patients proposed to benefit. Well-designed clinical trials will likely be necessary to know definitively whether the proposed therapeutic target is actually involved in the disease process, as opposed to parallel or downstream processes which cause morphological or biochemical changes without functional relevance.

To maximize the chance for success, basic neuroscience must be able to explain neurodegenerative diseases at all levels: molecular and cellular, neural tissue, and neural circuit (where the patient's neurologic deficits and burden of illness are manifestations of the neural circuit abnormalities). In this chapter, we highlight areas in basic science where major gaps in knowledge exist, review new technologies, and discuss anticipated challenges to clinical research in neurodegeneration.

Causation on the Cellular and Molecular Level

What is the nature of the most upstream causative link between protein mutations and the neurodegeneration they cause?

Neurodegenerative Diseases as Proteinopathies

"Culprit" Proteins

The key discovery in neurodegenerative disorders over the past decades has been the evidence for "proteinopathies" in almost all disorders. Abnormal processing of specific proteins occurs in neurodegeneration due to uncommon mutations in both the genes for specific proteins as well as in the common "sporadic" disorders: for example, proteins identified in dominantly inherited and sporadic diseases, including amyloid beta (Aβ) in Alzheimer disease (AD), tau in frontotemporal dementia (FTD), TAR DNA-binding protein 43 (TDP-43) in FTD or amyotrophic lateral sclerosis (ALS), and synuclein in Parkinson disease (PD). This led to the plausible idea that neurodegenerative diseases are caused by pathogenic effects of specific "culprit" proteins. Indeed, in each of these cases, the suspect protein has been found to misfold and aggregate, giving rise to a pathologic "signature protein" for each disease. Notably, most

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of the proteins involved in neurodegenerative disorders can exist in a dazzling array of conformations and assembly states; however, the most pathogenic effects of these conformations and states remain to be identified. This lack of knowledge has hindered both the development of treatments aimed specifically at the most pernicious culprits and the assessment of whether any of the treatments which have undergone clinical trials actually affected their levels in relevant brain regions.

Gain-in-function mutations in the genes coding for signature proteins are causal in several inherited forms of neurodegenerative diseases. This seems to offer an inherently important pathogenetic clue and enables experimental models to explore the molecular biology of neurodegenerative diseases using modern genetic techniques. A variety of cell function abnormalities have been identified in cellular and animal models expressing the mutant gene: some are related to abnormalities in pathways regulated by the normal protein, some to pathways unique to the mutant protein, and some to the transport and cellular location of the culprit protein. In general, though, the function of the normal protein is incompletely understood. The difficulty lies in determining the most upstream consequence of production of the abnormal protein versus the many downstream consequences that are expected in a diseased cell. This distinction is important as therapies aimed at a single upstream target may be effective, whereas a therapy aimed at only one of many downstream targets may not.

The temporal sequence of pathology may also be important. For instance, can early stages of pathology be driven by the abnormal protein but later stages of pathology be less dependent on the initial trigger? The idea of temporal stages has been advanced to explain the early failures of immunotherapy for AD patients. Is the disease in such a late stage in persons with dementia that removal of Aβ is no longer able to attenuate disease progression? Given this concern, there is a move toward earlier treatment and studies of asymptomatic persons with AD biology; that is, people known to have inherited amyloid precursor protein (APP) or presenilin mutations or who have been identified through positron emission tomography (PET) and cerebrospinal fluid (CSF) studies to be at high risk for developing AD. Amyloid PET scans, which show evidence of extracellular Aβ plaques before cognitive changes, may enable clinical trials in presymptomatic populations. Tau PET scans, which show evidence of intracellular tau aggregates, should enable detection and subsequent monitoring of the spread of neurodegeneration in AD as well as in the other tauopathies: progressive supranuclear palsy, FTD-tau, and chronic traumatic encephalopathy. The NIH planning groups for PD have recommended that new efforts be made to identify synuclein pathology years before onset of motor diagnosis. Major studies (PHAROS and PREDICT) identified premotor symptoms and imaging markers in Huntington disease to enable clinical trials that might prevent disease onset (Paulsen et al. 2014).

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Toxicity of Misfolded Proteins

The second intriguing idea dominating much of the current research follows from the evidence that in each of the neurodegenerative diseases, the culprit protein—whether in monogenic or sporadic disease—misfolds and forms aggregates within the cell (tau, TDP-43, synuclein) or outside the cell (Aβ). This has motivated the question, "Are neurodegenerative diseases disorders of protein misfolding and subsequent aggregation?" Considerable work is ongoing to determine if misfolded proteins are toxic to the cell, either as oligomers or full-fledged aggregates. Some studies suggest that the inclusion bodies filled with protein aggregates are a protective mechanism that walls off the toxic effects of monomers or oligomers. What are the important targets of toxic oligomers? Do they impair mitochondrial function, as has been purported to occur in PD? Biomarkers that allow measurement of these signature protein aggregates in humans can make profound contributions for diagnosis, the detection of presymptomatic onset, and the following of spread and propagation; they also can serve as targets for therapies aimed at decreasing production or increasing clearance. However, the identity of the most pathogenic conformation or assembly state is uncertain for most of the proteins causally involved in neurodegenerative diseases.

Impaired Proteostasis

The study of protein misfolding, due either to mutations in their coding sequence or simply overproduction, intersects with the cell biology of protein processing and metabolism (Tian and Finley 2012; Chen et al. 2011; Hamasaki et al. 2013; Hara et al. 2006; Nishida et al. 2009; Park and Cuervo 2013). Molecular chaperones shepherd normal protein folding in the endoplasmic reticulum and cytosol (Voisine et al. 2010). Does the production of mutant proteins or overproduction of "signature" proteins overwhelm the chaperone system, thus creating and resulting in widespread aberrant misfolding of the culprit protein (Komatsu et al. 2006; Koga et al. 2011; Orenstein et al. 2013)? Does this also lead to misfolding of other essential proteins in the cell? A number of mutations in protein-processing genes have also been causally linked to ALS and FTD: ubiquilin 2, vasolin-containing peptide, optineurin, charge multivesicular body protein 2, and vesicle-associated membrane protein-associated protein 2. The downstream effects of the production of misfolded proteins are an area of intense study. The molecular chaperone system preserves normal protein folding in stress conditions that promote denaturation. However, proteins that exceed the capability of the chaperones and become misfolded are metabolized by the ubiquitin proteasome system (UPS) as well as by chaperone-mediated autophagy. Larger aggregates are substrates for micro- or macro-autophagy into lysosomes. The fact that the culprit proteins form intracellular aggregates suggests that normal protein-processing mechanisms

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are not sufficient. Investigators are pursuing questions such as: Do culprit proteins in neurodegenerative diseases overwhelm these systems to the detriment of the cell? Do they create unsustainable endoplasmic reticulum stress and affect normal protein processing, lipid metabolism, and autophagy of other organelles (i.e., mitophagy of mitochondria)? The intersection of protein misfolding of culprit proteins and their effects on protein-processing systems also allows for consideration of a variety of other cellular circumstances to contribute to neurodegeneration. A disorder in protein processing might also explain the common co-occurrence of multiple "signature protein" aggregates in the same brain (i.e., Lewy bodies and AD pathology, TDP-43 and AD biology, the interaction of Aβ and tau in AD biology, and TDP-43 and tau in chronic traumatic encephalopathy). Indeed, one unanswered question is why these specific sets of proteins are so prominent among the entire set of intracellular proteins.

Banking on the possibility that deficiencies in protein metabolism may be common to neurodegenerative disorders, groups are testing whether agents which upregulate the capability of a cell's chaperone, UPS, or autophagic systems can attenuate the toxic effects of the "proteinopathies" in cell and animal models (Lee et al. 2010). New high-resolution confocal microscopy methods now allow investigators to follow fluorescently tagged proteins and organelles from their synthesis to degradation in cell models. Biomarkers that reflect the activity of autophagy or mitophagy in humans would enable testing this strategy in patients with a variety of neurodegenerative disorders.

Transmission and Propagation of Protein Misfolding

Over the past decade, evidence has accumulated that points to the propagation of misfolded proteins in the nervous system (Clavaguera et al. 2014a). In most neurodegenerative diseases there is spread of pathology from regions affected early (i.e., hippocampus in AD, vagal motor nucleus and olfactory bulb in PD, monolimb motor neurons in ALS, and dorsolateral tail of caudate in Huntington disease) to widespread involvement at late stages of disease. All brain regions do not degenerate synchronously. Spread and propagation of neurodegeneration was first identified in prion diseases. Recently, fibrillar aggregates of tau and synuclein have been seen to be taken up into otherwise normal cells and give rise to misfolding. Injections of fibrillar aggregates purified from human brain have caused aggregation of the corresponding mouse protein (Clavaguera et al. 2013; Watts et al. 2013). Local injection of mutated tau in viral vectors has been seen to cause tau aggregation in synaptically connected neurons that do not contain the virus, suggesting cell-to-cell transmission and propagation of misfolding (de Calignon et al. 2012). How are these proteins transferred from cell to cell? Some evidence suggests they are released from degenerating axons into the synaptic space. How are they transported in the cell to seed further misfolding? What processes are necessary for propagation of misfolding after entry of misfolded proteins? Are the current propagation models relevant

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to the human condition? Does the spread of misfolded proteins cause neuronal dysfunction or degeneration? Can transmission or propagation be blocked for therapeutic benefit (Clavaguera et al. 2014a)? Where in the nervous system is the site(s) of initiation? What is the initiating factor? Is it intrinsic to the neural cell? Is it triggered by some stressor coming from the vasculature, the nasal cavity, oral cavity, the gut, or the lung (Clavaguera et al. 2014b)? For example, in PD, synuclein fibrils may be seen in the fibers of the enteric nervous system that innervate the gut and parasympathetics that innervate the submandibular glands. Does synuclein aggregation begin in these peripheral nerve fibers and spread into the central nervous system?

Neurodegenerative Disorders as Errors involving RNA

Recent discovery of mutations in RNA-binding proteins in neurodegeneration (TDP-43, FUS/TLS, C9orf72, spinocerebellar ataxias) have shifted the focus to errors in RNA (Birman 2008; Ling et al. 2013; Ramaswami et al. 2013). Toxic RNAs have been postulated as causal features in ALS, FTD, and fragile X tremor ataxia. Some spinocerebellar disorders are due to mutations in noncoding RNA. In most cases of ALS and FTD, ubiquitinated TDP-43 is seen in neurons and glia, and it is common in AD as well. FUS inclusions have been found in Huntington disease, and spinocerebellar ataxia 1 and 2. Cytoplasmic and nuclear aggregates of RNA-binding proteins are seen in a number of neurodegenerative disorders, which may affect RNA homeostasis, translation of mRNA, sequester important RNAs and RNA-binding proteins, and affect transport with unhealthy consequences for the cell. Aggregates of RNA-binding proteins may also add stress to the cell's protein-processing capacity, thereby accounting for the co-occurrence of RNA-binding protein aggregates with aggregates of the "signature proteins." Abnormal gene regulation has been identified in a number of other disorders, including Huntington disease. The causative RNA mechanism in these disorders has not been definitively defined but includes affecting RNA processing and metabolism, gene transcription, spliceosome function, miRNA processing, and transport of RNA to specific cellular regions (synapse, axon). The role of noncoding micro RNAs in neurodegenerative disease has only recently started to be explored.

Neurodegenerative Disorders as Maladaptive Aging

As aging is an inherent component of all the disorders, any full picture of neurodegeneration must account for its role. Changes in the capability of cells, over time, to manage the stress response and process misfolded proteins in the UPS or autophagy systems, especially in nonregenerative, long-lived neurons, could provide the biologic link (Cuervo and Wong 2013). Aging might also be defined as an integral part of the cell's lifetime of protein stress. What are the ramifications for maintaining normal protein homeostasis for decades

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in the face of (a) a mutant protein, (b) overproduction of protein, (c) protein misfolding due to activation of Ca^{2+}-dependent proteases related to excitatory neurotransmission, (d) oxidative stress due to mitochondrial uncoupling and free radical production, (e) mechanical trauma, and (f) other yet unknown cell stressors? Aging science has identified a number of molecular and cellular processes of senescence, which should be considered as contributors to the "ticking clock" of neurodegeneration. In addition to aging processes in the neuron, aging effects at the tissue (i.e., vascular, glial, cerebrospinal fluid flow, or even whole body system) level (immune, metabolic) could contribute to the occurrence of dementia with age.

Mitochondrial Dysfunction

Accumulations of mitochondrial mutations and decreased mitochondrial function have been identified as contributing to neurodegeneration in most disorders as well as in aging itself. In some models, mitochondrial dysfunction is a consequence of the signature proteinopathies. Mitochondrial dysfunction is likely central to a number of neurodegenerative disorders as it is causally linked in Pink1 and Parkin mutations in PD (Burchell et al. 2013; Hertz et al. 2013), and superoxide dismutase mutations in ALS. The mitochondrial hypothesis has led to clinical trials of a variety of agents to improve energy metabolism and/or decrease oxidative stress in sporadic AD, ALS, PD, and Huntington disease. None of these trials (with the possible exception of CoQ10 effects on elevated brain lactate in Huntington disease) were accompanied by direct evidence that the drugs had any effect on energy metabolism in patients. Improved means of assessing mitochondrial function in brain are necessary to test the mitochondrial hypothesis.

Epigenetics in Neurodegenerative Disease

A variety of epigenetic changes with aging of the cell may result in changes in gene transcription that contribute to the age dependence of neurodegenerative disease. A relatively new idea of possible relevance is the finding that transposable elements in the genome become free to move as cells age and are able to reinsert into the genome with age (Perrat et al. 2013; Reilly et al. 2013). Possibly related to this notion are observations that Aβ accumulation increases the level of neuronal activity-induced DNA double-strand breaks, thus delaying their repair.

Compensatory Cellular Processes

Somewhat related to the study of additive effects of cellular stressors, it is known that a variety of "trophic" factors attenuate the effects of stress on cell health. Early studies sought to leverage the protective properties of single

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"growth factors" without full knowledge of their effects on the cells affected by neurodegeneration, and they did not meet with success. After acute brain injuries, there is almost always functional recovery over time associated with immune responses to injury, enhanced neurogenesis, movement of endogenous neuroprogenitors into the injured area, axonal and dendritic sprouting, and new synapse formation. What is the spectrum of compensatory processes in neurodegeneration? How are compensatory pathways affected by the proteinopathies? Do such compensatory processes attenuate the onset of pathology? Do compensatory processes deteriorate with age and does their rundown contribute to the requirement for aging in neurodegenerative diseases? Would enhancement of these compensatory processes be expected to attenuate disease progression? On the other hand, does the likelihood that evolution has already optimized compensatory processes limit the therapeutic potential of strategies aimed at their enhancement? Furthermore, could these compensatory processes go array, as occurs in posttraumatic epilepsy, and result in epileptiform activity that worsens neurodegeneration?

Neurodegeneration and Neural Cellular Dysfunction: Neurons and Glia

Cell death is common to the neurodegenerative diseases, but cell dysfunction is expected to occur long before a cell dies. Less studied than the molecular/organelle dysfunction in neurodegeneration is the function and structure of the neuronal components—such as the axon (Morfini et al. 2009; Yue et al. 2008), dendrite (Stephan et al. 2013), and synapse—or how, under what circumstances, the molecular abnormalities affect the neuron's ability to function in its network. At what stage do the molecular abnormalities underlying neurodegenerative diseases affect the neuron's ability to function in its network? How does this affect the circuit function? Aggregates are seen in many neurodegenerative diseases in glial cells (tau in tufted astrocytes in progressive supranuclear palsy, synuclein in oligodendrocytes in multiple system atrophy) but little is known about their effects on glial cell biology or glial functions. This gap in knowledge is highlighted by the finding that mutations confined to microglial cells or glial cells in genetic mouse models can have profound neuropathological effects. In a neuroncentric way of thinking, these effects are called non-cell autonomous.

Genomics of Neurodegeneration

Although there has been considerable progress in understanding the genetic underpinnings of neurodegenerative disease, much more can be learned with the newer techniques that are now available. Genomic studies on brain tissue have become especially important to study epigenetic signatures of neurodegenerative diseases. A new area of study, understanding whether mosaicism contributes to neurodegenerative disease, has been ushered in by the discovery

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that somatic mutations, especially copy number variants, are enriched in the brain. Whole exome sequencing also allows the fairly rapid discovery of *de novo* mutations in studies of trios (mutations in affected individuals not found in unaffected parents). Genomic studies of brain tissue also allow the tracking of insertions by large volumes of transposable elements in a cell's DNA, which may escape epigenetic processes that hold these in check, whether due to age-related changes in histone modification or epigenetic events.

Extrinsic Triggers of Cellular Degeneration

Advances in genomics have opened a great new knowledge base in neurodegenerative disease. Much less is known, however, about the causal influences that are extrinsic to the cell. For instance, excitotoxicity was long studied as a common stressor that might lead to neurodegeneration. Epileptiform activity has recently been identified in AD as a potential contributory factor. The identification of environmental influences that affect the development of neurodegeneration remains a knowledge wasteland. There is some evidence that pesticide exposure links to PD; ALS was seen at slightly higher frequency in military personnel after the Gulf War; and repetitive concussion has been linked to a neurodegenerative tauopathy as well as to motor neuron disease. It has been suggested that concussion is a risk factor for AD, but that risk has not been clearly defined. The interaction between vascular disease and AD is complex, since they co-occur commonly in persons with dementia, and vascular risk factors are highly correlated with risk for dementia. A variety of research has tied stressors or "second hits" to neurodegenerative disease, but whether they are one of thousands or an important influence by themselves is not clear. Of interest, some protective factors have been identified from epidemiologic studies (e.g., caffeine, brain penetrant calcium channel blockers, and high urate levels) to decrease risk of PD. Because techniques are not available to measure lifelong exposures to chemicals, microbes, oxidative stress, metabolic stress, mechanical insults, or even brain circuit activity, the environmental and extrinsic influences which likely contribute to development of neurodegenerative disease may remain largely unknown and difficult to study.

Systems Biology of Neurodegeneration

Some investigators highlight the need for a systems approach that interrogates many, if not all, molecular pathways within the cell to avoid the pitfalls of focusing on a single pathway and not appreciating how it is integrated into tens or hundreds of others. A cell's state may be defined by networks of interconnected proteomic, metabolomic, and transcriptomic patterns. No single pathway aberration is sufficient to cause neurodegeneration, but specific "network states" may underlie neurodegeneration. The therapeutic target, then, becomes

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normalization of the cell's abnormal network "state," as opposed to intervening in a single pathway.

Tissue Level Dysfunction in Neurodegenerative Disease

Neurovascular Unit and Glymphatics

Some argue that the current and past approaches to neurodegenerative disease have been overly neuroncentric. The neuron functions within a dense cellular tissue made up of different types of glia, resident inflammatory cells, hematopoietic inflammatory cells, various cellular, and matrix components of the vasculature. A recent term from vascular biology is the "neurovascular unit." In addition to the coupling of blood flow with neural activity, control of movement of cells, and molecules across the blood-brain barrier, another system (called the glymphatic system) has been identified which parallels the lymphatic system in other tissues (Iliff et al. 2012) The fluid movement in the interstitial space is dynamic, as is the movement of cerebrospinal fluid from production in the choroid plexus to absorption into the blood stream via the arachnoid villi. Most recently, the flow of interstitial fluid through the glymphatic system has been ascribed a role in the "flushing" of proteins such as Aβ from the brain, especially during sleep. Interstitial fluid is described to enter from the cerebrospinal fluid (CSF) space along penetrating arterioles, pass through glia, and exit along perivenous channels back to the CSF. Aβ clearance from brain has been identified as a potential contributor to amyloid plaque burden. Unexplored remain how glymphatic function changes with age, the distortion to brain structure that occurs with aging, hypertension, brain atrophy, and changes in CSF flow (e.g., enlarging ventricles, function of meningeal tissue, geometry and flow in widened subarachnoid space, arachnoid granulations affecting CSF absorption, choroid plexus aging and CSF production).

Vascular Disease and Alzheimer Biology

In persons dying with dementia, a combination of vascular diseases (gross infarcts, micro infarcts, diffuse white matter disease) commonly occurs with AD pathology. Indeed some data suggests that their combined burden of pathology leads to the most common forms of dementia. Vascular risk factors, most prominently the presence of infarcts, are one of the strongest risk factors for dementia of the AD type. Of note, the predominant site of infarction is in deep white matter and basal ganglia nuclei, not cortex. The nature of the association of mixed dementia with diffuse white matter disease (a mix of infarction, wide periarterial spaces, rarefaction of myelin) is also unknown. New data suggests that metabolic syndrome (obesity, type 2 diabetes, sedentary lifestyle) is associated with dementia, but whether this is via effects on vascular disease or

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some hormonal effect of the abdominal fat pad or a combination of factors is not clear.

Vascular function and the biology of AD are intertwined. Aβ clearance into the blood has been shown to rely on normal function of the blood-brain barrier which may be disordered with age and hypertensive vascular disease. Aβ actually appears to be toxic to pericytes, which are important regulators of the blood-brain barrier. Aβ amyloid deposits in the blood vessel wall in a large percentage of persons with AD and affects blood-brain barrier function, even giving rise to multiple hemorrhages in many (amyloid angiopathy). How dysregulation of blood flow, oxygen, glucose delivery to tissue, and blood-brain barrier function contribute to AD pathology is not entirely clear.

Functional Consequences of Gliosis and Inflammation in Neurodegenerative Disease

Gliosis is ubiquitous in neurodegenerative disease yet little is known about glial function or how it may contribute to progression in these disorders. In some neurodegenerative disorders, the signature protein misfolding occurs in glial cells (multiple system atrophy, chronic traumatic encephalopathy). In progressive supranuclear palsy, tau fibrils are seen in oligodendroglia; how that affects axonal transmission of action potentials is, however, not known. Much more is becoming known about the normal biologic interactions between glia and neurons that might now be explored in disease. Inflammation is ubiquitous in most neurodegenerative diseases. The role of inflammatory cells, however, is now known to be complex (Glass et al. 2010) and has recently been identified as essential to the process of normal synaptic pruning (Stephan et al. 2013). They are also involved in regulating the movement of neuroprogenitor cells along vascular pathways. Neuroprogenitor cells in the dentate, periventricular stream, and nasal mucosa may have a role in repair in the nervous system, but we know little of their role in combatting neurodegeneration. Cellular inflammation and cytokine secretion occurs in ischemic vascular disease and may be one of the links between cerebrovascular disease and neurodegeneration. The tissue processes that contribute to or combat cell-to-cell transmission of culprit misfolded proteins is an acute area in need of research.

Reparative Tissue Responses in Neurodegeneration

Reparative processes at the tissue level are also mainly unexplored. Pathologic studies, in most cases, indicate that the brain tissue structure is abnormal by the time symptoms occur. Whether this hysteresis is related to some threshold of pathology required to disturb neural circuits or whether there is some reparative process that temporarily compensates for the neurodegeneration is currently not known. Some of the early cellular and molecular changes may be related to compensatory changes at the tissue level, as opposed to contributors

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to pathology. For example, protein aggregates have been shown in some cases to have protective attributes by preventing the harmful activity of toxic oligomers. Microglia have important normal functions in pruning ineffective synapses. The protective effects of gliosis are not clear. In general, we know little about the tissue function level in neurodegenerative disease.

Circuit Level Dysfunction in Neurodegenerative Diseases

Behavior and neurological deficits which characterize neurodegenerative diseases are considered manifestations of neural circuit dysfunction. In the past, correlation of neurologic deficits with pathology pointed to the circuit dysfunction in neurodegenerative diseases (i.e., semantic memory dysfunction with pathology in hippocampal and cholinergic regions, progressive aphasia with focal cortical atrophy in language cortex, loss of visuospatial skills with right parietal atrophy, bradykinesia with ventral substantia nigra). Most recently, new technologies are allowing dynamic assessment of neural circuit function, such as resting state magnetic resonance imaging (MRI), graphic analysis of electroencephalography (EEG), magnetoencephalography, or in response to specific tasks. These techniques have provided evidence for signatures of circuit dysfunction for specific disorders and are being validated as a means to measure progression of circuit dysfunction over time. This work is at an early stage. As its final product, a comprehensive predictive model of neurodegenerative disease would explain neural circuit abnormalities and exactly how these translate to neurologic dysfunction. If known, then tracking changes in circuit function would enable dynamic monitoring of progression and provide targets for therapy that are tied closely to the features important to patients. In addition, circuit tracking might enable answers to an important conundrum in neurodegenerative disease: the lack of perfect correlation between burden of pathology and burden of illness.

One explanation is that we cannot actually see or measure the full burden of pathology. Another is that individual variation exists in how neural circuit function is affected by neurodegenerative changes at the tissue level. Studying this problem might lead to an understanding of the mechanisms underlying "resilience." Studies of the healthy "very old," for instance, show good cognitive function despite presence of AD pathology. Is this due to compensation at the molecular or tissue level, which preserves circuit function, or are other circuits called in to compensate for dysfunction in the usual disturbed circuits? Is it possible to alter neural circuits in neurodegenerative disease to improve function? Mental activity, physical exercise, and educational level have been suggested to attenuate or delay neurodegenerative disease. Suppression of network hyperactivity by treatment with an antiepileptic drug has been shown to improve cognitive functions in a mouse model of AD and patients with

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amnestic mild cognitive impairment. Are these interventions altering circuit function in some beneficial manner related to or independent of the pathobiology at the tissue level? Deep brain stimulation certainly produces profound benefits for motor function in PD, but has had no effect on disease progression.

New Technologies

Many of the questions posed above are not novel and have been pursued in some manner for decades. For instance, transmission and propagation of misfolded proteins was tested by Carleton Gadjusek in the last century. New technologies often lead to breakthroughs in testing key hypotheses. New technologies also allow measurements of things never seen before, which in turn opens up entirely new hypotheses. Certainly, many of the advances over the past decade come from impressive new capabilities in human brain imaging, computer analysis of large data sets, genetics and genomics, molecular imaging, and cellular imaging. Looking to the future, one might expect continued major improvements and the introduction of new technologies. One objective of the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative in the United States is to expand the power of these types of tools and develop others for interrogation of neural circuits.

Interrogation of Neural Circuits

In circuit analysis, intracranial recordings have been of value in understanding abnormal circuit function in small, defined areas in patients with epilepsy, and advances will allow greater resolution and coverage of brain. Genetically engineered light emission technologies linked to changes in intracellular calcium and membrane voltage now allow simultaneous recording of thousands of neurons. This technology may be expanded to monitor a variety of other signaling processes in animal and cellular models (e.g., light emission linked to enzyme activity, organelle function, channel activation, protein aggregation). A new technique to extract lipid to make volumes of brain accessible to light microscopy will allow rapid three-dimensional analysis of molecules and structure. Human brain circuit analysis is under intense study using the BOLD (blood oxygenation level dependent) technique, EEG and evoked potentials, and magnetoencephalography. Each of these has inherent limitations in terms of temporal or spatial resolution and the accessibility of deep brain structures, which completely new technologies will be required to overcome. It may occur that exquisitely sensitive and specific intracerebral techniques will be developed to record circuit behavior in animals and animal models of disease; however, their invasive nature does not allow use in humans. Developing safer means of recording from inside the skull in humans may rise as a major challenge to translate the advances made in animals.

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Fiber Tracking in Neurodegenerative Disease

White matter atrophy in neurodegenerative diseases is known to be quite marked, but little is known about the specifics of the loss or the effects on neural circuits. Efforts, such as the NIH connectome project,[5] in charting the white matter connections of the human brain in thousands of individuals using diffusion MRI will likely enable a new field of exploration in neurodegenerative diseases that parallels current and past studies of regional gray matter loss. Though studies of white matter structure in neurodegenerative diseases may expand, there is still only crude technology to interrogate white matter function (i.e., focal transcranial magnetic stimulation coupled to EEG or fMRI to detect target activation).

Induced Pluripotent Stem Cells

Scientific progress is often dependent on the available experimental models and their ability to dissect mechanisms important in disease. Transgenic animal models have transformed the field over the last few decades but as yet have not predicted therapeutic success in human trials. The advances in induced pluripotent stem cell (iPSC) technologies, which are only a few years old, allows experimentation to move to human cells (Bilican et al. 2012, 2013; Ryan et al. 2013; Serio et al. 2013). In just a short time frame, iPSC technology has provided the opportunity to establish specific phenotypes in cells from individuals with genetic causes of neurodegeneration (with the abnormal gene corrected in clonal iPSCs as controls) and to study the mechanisms underlying those phenotypes. The key issue is to discern which phenotype, discovered in iPSC cultures, proves to be relevant to disease pathogenesis. Though initially studied *in vitro,* the transplantation of iPSCs into an *in vivo* environment in animals may allow important observations about their interaction within the tissue and neural circuits and the effects of aging. Whether iPSCs from patients with sporadic disease will also manifest valuable specific phenotypes is yet to be definitively determined. An event that occurs in a spinal motor neuron or enteric nerve fiber due to an environmental stressor may not be shared by a skin fibroblast. Replacement therapy in human patients is an ultimate goal and may be on the horizon in focal atrophies, such as dopaminergic cell replacement in PD. Whether iPSCs themselves can be used to deliver trophic factors to attenuate neurodegeneration widely in the brain is not clear.

Manipulation of Genes and mRNA

Though genetic techniques are prominent in cellular and animal studies, gene therapy in human neurodegenerative diseases is still in its infancy. Delivery

[5] http://www.humanconnectomeproject.org/ (accessed April 9, 2015)

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problems are being worked on using a variety of viral vectors and direct delivery into brain or CSF of "protected" iRNA and antisense. If toxicity can be managed and delivery to the necessary intracellular sites is efficient, it is conceivable that knockdown of gain-in-function mutations will effectively treat the autosomal dominant neurodegenerations. A variety of other genetic manipulations then become possible in non-Mendelian neurodegenerations, including decreases in synthesis of "culprit proteins," and upregulation of protein clearance mechanisms such as autophagy-lysosome systems or proteasome-ubiquitin systems. Similar issues face antibody therapy, which is currently being tested to improve clearance of extracellular accumulation of misfolded proteins. Antibody therapy has been postulated as a potential treatment to prevent cell-to-cell transmission of protein misfolding.

Advanced Genomics and Proteomics

Molecular techniques to assay large numbers of genes, noncoding DNA, histone modifications, and mRNA are becoming more sophisticated at a tremendous rate and should enable the study of gene networks, epigenetics, mosaicism, and the movement of transposable elements in a cell's genome using sophisticated computational techniques. iRNA screens can be used to identify the complete set of interrelated genes. Proteomic technologies that couple mass spectroscopy to antibodies, DNA, or protein aptamers are also able to detect femtomolar quantities of thousands of known proteins. These will allow the study of protein networks in models of neurodegenerative disease, such as iPSCs, but can also be applied to measure important signatures of disease or pharmacological targets in cerebrospinal fluid. This field could be advanced by a concerted effort to characterize the normal CSF proteome across the life span.

PET Radioligand Development

PET tracer development is an intense area of investigation, and even commercialization now, to detect and follow protein aggregation in certain neurodegenerative diseases. Improved tracers promise to transform diagnosis of disease in the presymptomatic stages and provide biomarkers of disease progression. Radioligands that bind to extracellular amyloid and intracellular tau aggregates as well as inflammatory cells have been incorporated into ongoing clinical studies. Efforts to find radioligands for synuclein imaging are underway.

Computational Techniques and Big Data

A number of groups have questioned whether science could be advanced by a data repository for clinical and basic research data. Would such a large database

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lead to discoveries from the application of analytic tools to "big data"? The density of data accumulated in genomic, proteomic, neuroimaging, and neurophysiology studies requires sophisticated analytic techniques to identify important relationships. Certainly the lack of foresight necessary to combine data from the many large and expensive ongoing studies around the world appears wasteful.

Anticipated and Current Challenges to Clinical Research in Neurodegeneration

How to Approach the Environmental Determinants of Neurodegeneration

Probably the most difficult challenge in understanding and preventing neurodegenerative diseases is to identify those factors across the life span of the individual that either increase or decrease risk (Rodriguez-Navarro et al. 2012). It is likely that we will know the genetic load within decades, but even then we will struggle to account for the environmental load that leads to non-Mendelian neurodegeneration, which affects the majority of individuals. Though genetics has advanced enormously, the ability to identify causal "environmental" risk factors to measure their effects in brain and to develop methods to mitigate their effects over time is almost nonexistent. Epidemiologic studies sometimes highlight particular correlations: traumatic brain injury and vascular risk factors in dementia, pesticides in PD, and protective effects of caffeine in PD. They suffer, however, from lack of precision in measurement, abundance of confounders, and purely correlative relationships to disease. The fact that these exposures may occur decades before disease onset makes study very difficult. It may be possible and insightful to approach the gene-environment problem by studying persons with defined genetic risk and attempt to uncover the environmental factors that affect penetrance (i.e., LRRK2 PD or apoE4 homozygotes).

Bioethical Issues Associated with Ultra-Early Detection of Neurodegenerative Biology

Current thinking in neurodegenerative diseases also places importance on early detection, before symptoms of disease are evident by neurologic examination. This is now possible in AD with technologies (amyloid PET imaging and CSF Aβ/P-tau ratios) to identify and measure the pathology that precedes symptoms. Efforts are underway to replicate this in other neurodegenerative disorders. This new technology raises significant ethical questions which must be resolved culturally to proceed: How should investigators ethically recruit and inform individuals at risk for neurodegeneration in the future? How should these individuals be supported by researchers and the medical system? How is privacy of "risk" to be maintained? In designing interventions, what ratio of

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risk/individual benefit is appropriate in trials of asymptomatic individuals, especially with variable degrees of penetrance, variable confidence in estimates of risk, etc.? How should we ethically manage expectations in trials of asymptomatic individuals?

Biomarkers for Therapy Development in Neurodegenerative Diseases

Perhaps the most acute challenge to neurodegenerative disease research, as well as therapy development in other CNS disorders, is the failure of preclinical models to predict success in clinical trials. This may be a factor of the limitations of the models but it may also be an indictment of the clinical trial strategies. It has been difficult to demonstrate that a particular treatment has the intended molecular effect in humans at the implemented dose and duration plan. Current thinking puts highest priority on developing the means to test for target engagement in human brain, proof of principle that a therapy has the expected biologic effect at feasible doses and delivery strategies. The science of discovery and validation of useful measurement of target engagement in neurodegenerative diseases requires renewed effort. Particular gaps exist at the validation stage of a biomarker of disease pathophysiology. Numerous reports of measurements that differ in small numbers of patients versus some control group occur in the literature, but very few ever become useful for research. Lacking is the standardization of the measurement, study of confounders, assessment of variance in the measure from the same individual over time (test-retest variability), measurements from different populations with the same disease (site to site variability), and understanding changes that occur with changes in techniques, reagents, or different scanners, etc. These problems plague current techniques such as task-dependent fMRI, resting state MRI, diffusion imaging of white matter tracks, CSF proteomic studies and multiple genomic studies. Academic investigators are unlikely to solve these problems by themselves. Since useful biomarkers for neurodegenerative disease research are necessary, partnerships between academics, industry, and government will be required.

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