Life in the Fast Lane: The Journey from Squid Axons to Alzheimer’s Brains

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Axons became swollen on the proximal side of a crush injury and degenerated when separated from the neuronal cell body.
Bulk "flow" of axonal materials moved down the nerve at a rate of 1-2 mm/day.
Enzymes associated with neurotransmitter synthesis moved in both directions
Waves of radioactively labeled proteins were moved down the nerve at a rate of 200-400 mm/day

Raymond Lasek
1967
Neurotransmitters and the vesicles that contained them were being transported from cell bodies to synapses at >100 mm/day on microtubule tracks.
Robert Allen’s development of Video-Enhanced Contrast Microscopy allowed us to see this movement for the first time in living neurons.
Anterograde Fast Axonal Transport
1.5-1.8 µm/sec

Retrograde Fast Axonal Transport
1.1-1.4 µm/sec
A novel brain ATPase with properties expected for the fast axonal transport motor

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A key question remained: How do you target cargos to the right location?
Kinases and Phosphatases were likely candidates to regulate Fast Axonal Transport.

There are more than 500 genes encoding for kinases in the human genome, so we began to test many of the most common ones in axoplasm.
We began to introduce purified enzymes into the axoplasm and soon found that different kinases had different effects on Fast Axonal Transport.
We’ve gone from Squid Axons to Molecular Motors and begun to understand regulation of fast axonal transport.
How do we get from axonal transport and squid axons to Alzheimer’s brains?
Adult-Onset Neurodegeneration

FTD
Location: frontotemporal
Macro: cerebral atrophy
Micro: tau deposits, Pick bodies

LBD
Location: frontotemporal
Macro: cerebral atrophy
Micro: Lewy bodies

HD
Location: basal ganglia
Macro: neostriatal atrophy
Micro: neuronal loss and astrocytosis

Prion disease
Location: diffuse cortical
Macro: cerebral atrophy
Micro: spongiosis, PrP deposits

AD
Location: temporoparietal
Macro: cerebral atrophy
Micro: Aβ plaques, tangles

PD
Location: midbrain
Macro: pallor of substantia nigra
Micro: Lewy bodies

ALS
Location: motor cortex, brainstem, spinal cord
Macro: atrophy of motor neurons and muscles
Micro: inclusions (Bunina bodies, Lewy body–like)

Neurological diseases are defined by loss of connections: when the connections are lost, disease symptoms appear even if the neuron is still there.

Neurons rely on communication with their targets to survive. When they lose enough connections, they die, but the symptoms of a neurological disease appear years before the affected neurons die.
Each of these diseases can be related to different misfolded proteins and each has a signature pathology with unique pathological hallmarks.

What can we learn about the molecular basis for neuropathologies associated with misfolded proteins?

What makes misfolded proteins bad for neurons?
Adult-Onset Neurodegenerative Diseases

- Asymptomatic during development and maturation of the nervous system
- Both hereditary and idiopathic forms may exist with the same pathology
- Symptoms slowly progress for years after appearance of symptoms
- Misfolded proteins are a common feature of both familial and idiopathic forms
  - Projection neurons are affected primarily or exclusively
  - Select neuronal populations are affected and most neurons are spared
  - Affected neurons exhibit altered patterns of phosphorylation
- Altered synaptic function is an early feature of the disease process
- Neuronal loss proceeds with the characteristics of a dying back neuropathy
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Prevalence of Alzheimer’s Disease (AD) in the U.S. 1995 – 2050

Dementia in the USA

- Alzheimer’s Disease: 56%
- Multiple Causes: 12%
- Parkinson’s Disease: 8%
- Brain Injury: 4%
- Other: 5%
- Stroke: 15%

AD Cases

- 10% of people 65+
- 50% of people 85+

Graph shows the increase in AD cases from 1995 to 2050.
Percentage Change in Leading Causes of Death From 2000 to 2005

- Alzheimer’s: 44.7%
- Heart Disease: -8.8%
- Breast Cancer: -0.8%
- Prostate Cancer: -4.9%
- Stroke: -14.4%

In the Alzheimer brain:

- The **cortex shrivels up**, damaging areas involved in thinking, planning and remembering.
- Shrinkage is especially severe in the **hippocampus**, an area of the cortex that plays a key role in formation of new memories.
- **Ventricles** (fluid-filled spaces within the brain) grow larger.

Plaques and tangles (shown in the blue-shaded areas) tend to spread through the cortex in a predictable pattern as Alzheimer’s disease progresses.

The rate of progression varies greatly. People with Alzheimer’s live an average of eight years, but some people may survive up to 20 years. The course of the disease depends in part on age at diagnosis and whether a person has other health conditions.

- **Earliest Alzheimer’s** – changes may begin 20 years or more before diagnosis.
- **Mild to moderate Alzheimer stages** – generally last from 2 - 10 years.
- **Severe Alzheimer’s** – may last from 1 - 5 years.

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Alzheimer’s Disease

First recognized as a distinct disease in 1901 by Alois Alzheimer in Frankfurt, Germany based on his examination of a 51 yr old woman, Auguste D, admitted with signs of dementia. Her case was formally presented as “A Characteristic Disease of the Cerebral Cortex” in 1906.

In this paper, he described the distinctive clinical features of the disease including memory and cognitive deficits leading to loss of judgement, language and dementia. Alzheimer also reported the distinctive appearance of what are now known as plaques and tangles based on his histological examination of her brain. Based on age of onset, this was the first description of familial Alzheimer’s disease.
Solomon Carter Fuller was born in 1872 in Liberia, coming to the United States in 1889 to attend Livingston College in Salisbury, N.C., and graduating from Boston University Medical School in 1894. He then took a position at Westboro State Hospital and served as a faculty member at BU Medical School from 1919 until his death in 1953. He is considered to be the first black psychiatrist in America.

In 1904, Fuller went to Munich, Germany to study psychiatry in the laboratory of Alzheimer. In 1907 the American Journal of Insanity (AJI, later American Journal of Psychiatry), published his "Study of Neurofibrils in Dementia Paralytica, Dementia Seniles, Chronic Alcoholism" which confirmed the earlier results of Alzheimer and represented the first description of sporadic Alzheimer's disease.

In 1911 AJI published his paper on plaques in the brain of aged people, which stated, "The plaques were the deposits in brain tissue of a chemical substance resulting from pathological metabolism of nervous elements." In a later paper he used the term "amyloid." The Journal of Nervous and Mental Diseases published his report of the ninth case of AD in 1912.

-adapted from Lucy Ozarin in Psychiatric News (2002) 37:19
Hallmarks of Alzheimer's Disease

- Adult onset of symptoms (a disease of the aging brain)
- Selective vulnerability of neurons (many neurons and non-neuronal cells are spared)
- Accumulation of Aβ peptide oligomers and fibrils (placques)
- Accumulation of neurofibrillary tangles (filamentous inclusions comprised largely of tau)
- Altered phosphorylation of neuronal proteins.

Alzheimer's Disease
Down's Syndrome
Chronic Traumatic Encephalopathy
Primary Age-related Tauopathy
Guam Parkinson Dementia Complex

Tauopathies (tau mutations)
Corticobasal Degeneration
FTDP-17
Pick’s Disease
Progressive Subcortical Gliosis
Progressive Supranuclear Palsy*

Other Tauopathies
Agyrophilic Grain Dementia
Meningioangiomatosis
Postencephalitic Parkinson’s disease
Subacute sclerosing panencephalitis

All these have tau filament pathology. Tau filaments are clearly bad for neurons, but the question is why?

Horowitz et al., J. Neurosci. 24(36):7895-7902
Wischik et al. J. Cell Biol. 100:1905
Tau monomer has no effect on fast axonal transport, but tau filaments selectively inhibit anterograde, kinesin-based transport.
Activated PP1 dephosphorylates and activates GSK3β, which phosphorylates kinesin light chains and inhibits anterograde FAT.
The N-terminus of tau is necessary to activate the phosphatase and GSK3 kinase activities.
Alzheimer’s Disease and Tauopathies
When tau is misfolded, such as in neurofibrillary tangles, an N-terminal domain is exposed that activates a PP1/GSK3 signaling pathway, which inhibits kinesin-based anterograde transport by phosphorylating kinesin light chains  

Alzheimer’s Disease
Aβ oligomers, but not filaments activate CK2 and inhibit both directions of FAT. The effects are blocked by a conformation sensitive antibody.  
Pigino et al., Proc Natl Acad Sci U S A 106, 5907 (2009)

Huntington’s Disease
Pathological expansion of a polyQ tract in huntingtin beyond 36Q’s exposes a proline-rich domain that activates a JNK3/MAP kinase signaling pathway that inhibits both anterograde and retrograde fast axonal transport.  
**Amyotrophic Lateral Sclerosis**
Both mutant SOD1 and misfolded Wild Type SOD1 activate a P38 MAPK and inhibit anterograde FAT. The effects are blocked by a series of conformation sensitive antibodies, specific kinase inhibitors or chaperones.

**Parkinson’s Disease**

**Hereditary Spastic Paraplegia SPG4**
Mutations in a long isoform of spastin expressed in spinal cord inhibit both directions of FAT by activation of CK2, while these mutations in a shorter, more widely expressed isoform of spastin missing the first 86aa have no effect.
Solowska et al., J Neurosci 28, 2147 (2008)
Dysferopathy

from the Greek “φερο” or “fero”
meaning “to carry” or “to transport”

We propose the term “dysferopathy” to describe pathologies associated with compromises in FAT that lead to a late-onset, dying-back neuropathy.
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