

## Enabling Technologies 2004 Workshop Summary

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This report summarizes discussions and recommendations made at the fourth annual workshop on Enabling Technologies for Alzheimer's disease (AD), held in August 2004 in Bar Harbor, Maine. Academic and industry scientists from inside and outside the field of Alzheimer disease research met with foundation representatives and an administrator from the National Institute on Aging. Their goal was to identify knowledge gaps that are limiting progress in AD diagnosis and therapy, as well as strategies to bridge these gaps. On the first day, participants reviewed current knowledge on synaptic and axonal dysfunction in AD, and on protein misfolding in neurodegenerative diseases. The second day saw discussion of imaging frontiers in AD diagnosis and treatment monitoring.

Participants generated a table summarizing what findings have achieved consensus across the field and on which issues researchers still disagree. The points of discussion and final recommendations presented in this report emerged during the discussions and do not reflect an order of priority or the unanimous views of all participants. We'd love to hear your thoughts.

What We Know	What We Don't Know
APP's sequential cleavages. APP and PS mutations that increase Abeta42 cause familial AD	What regulates the secretases?
APP cleaves into other fragments, including AICD, C31 (via caspase cleavage), C100, etc.	What does APP do? What does A $\beta$ do? What about the other cleavage products?
A PS1 mutation causes a frontotemporal dementia without A $\beta$ deposition	Do PS1 and APP mutations cause dementia by pathways other than A $\beta$ ?
PS1 has many other substrates besides APP	Do these other PS targets contribute?
	Are there other enzymes that generate A $\beta$ ?
	What are the identities and functions of APP-interacting proteins?
	APP ligands? (Other than F-spondin and APP.)
ApoE only proven LOAD risk factor, affects age	Other late-onset genes (model

of onset.	suggest 4-6 others exist)
ApoE affects not production but clearance/deposition of A $\beta$ .	Precise mechanism of ApoE effect on A $\beta$ .
Enzymes can degrade A $\beta$ .	Role of IDE, neprilysin in AD; identity/function of other players in A $\beta$ degradation.
Aggregation of tau occurs, is damaging.	Mechanism of link between A $\beta$ and tau
Abnormal tau destabilizes microtubules.	Details of tau toxicity.
Synaptic dysfunction and loss occurs.	How and when? Where does it fit into the cascade of AD pathology?
First symptoms are impaired memory and executive function, change in personality.	
Classic pathology begins years before symptoms or detectable change in neuropsychology tests.	Are there changes that precede A $\beta$ pathology?
Hippocampal formation, parts of limbic system begin to atrophy, whole brain shrinks	Which cortical areas are affected first?
Changes in brain imaging, decreased activation in medial temporal lobe	Relationship between medial temporal and posterior areas.
Early onset disease is variable, late-onset disease is predictable	Is AD one disease or many?
Inflammation plays a role	When and how important?
Cell cycle reactivation	Ditto
Autophagy/endosomal lysosomal changes	Ditto
Gliosis	Ditto
Lifestyle factors protect but no agreement on which ones. Candidates include activity, education, diet, and antioxidants.	
Risk factors: aging, head trauma	Why is aging a risk factor?

## Major Points of Discussion and Ideas for Future Directions

### Axonal Transport

Larry Goldstein, of University of California, San Diego, laid out his hypothesis that defects in axonal transport could starve the nerve terminal of needed supplies and lead to synaptic dysfunction in AD, Huntington's, and perhaps other neurodegenerative diseases. On AD, Goldstein's work includes the proposition that APP functions as an anchor for the anterograde motor protein kinesin, and that BACE, PS, and APP travel down the axon together in vesicles, where APP cleavage occurs, particularly when traffic stalls. These latter aspects remain controversial but the participants considered the overall hypothesis plausible and recommended testing it further.

On axonal transport, current challenges include taking the fly and transgenic mouse experiments into the human. A practicable approach would be to ask whether there are peripheral effects, or effects in the narrowest fiber types. C fibers in skin are particularly amenable to study. Physicians do not generally observe widespread peripheral problems in people with early AD, but sensory problems have been reported occasionally in early AD and deserve further study in regard to axonal transport. Collections of fixed biopsy samples exist and might contain evidence of defects in synaptic vesicles and synaptic terminals. Peripheral neuropathies that might be related to axonal transport are common in the elderly. They have not been linked specifically to AD but are commonly seen in Parkinson's, which features a range of under-appreciated symptoms besides dopamine-dependent motor disturbances. In cell culture, neurons take up beads, transport them down axons, and exhibit bead-induced blockages. Can beads induce tau pathology, neuronal death, any form of disease?

Another challenge worth tackling lies in developing techniques to image axonal flow in humans. Initial approaches exist, but a major challenge concerns how to model the slow rate of change that may underlie AD. A one percent annual loss could become critical over 30 years, but compressing the process into days or weeks yields crude models. Surrogate markers for axonal transport ought to be developed, as they could serve as tools to monitor a mild, long-term defect in transport and become useful diagnostic tools. A genetic way to assess the slow rate of change in axonal transport would be to cross NINDS-funded mice that are transgenic for various synaptic or axonal transport proteins with existing APP transgenic mouse models in which a reduction of synaptic transmission can be detected.

### **Synaptic Dysfunction**

There was no agreement on how synaptic dysfunction relates to the development of classic pathologies, and on which drives which. One group held that abundant data suggest synaptic dysfunction is the earliest cellular manifestation of disease, others pointed out that YAC mouse models of AD do not show early synaptic/LTP changes. There was agreement that it is technically possible, and critical, to move past descriptive studies of synaptic changes and toward functional ones. For example, Frank LaFerla's model should be checked for synaptic changes prior to and in response to A $\beta$ -antibody treatment.

At this point, information about synaptic loss is available but insufficient to make it approachable as a therapeutic target. The heterogeneity and large number of homeostatic pathways at work in synapses and synaptic networks makes broad-acting drugs unlikely to improve memory; more specific synaptic defects must first be found. Then, prevention of that process should take priority over encouraging sprouting or other efforts to restore lost synapses.

The field needs imaging markers for synaptic dysfunction, some of which may also signal axonal transport deficiencies. For example, radioligands for markers of synaptic dysfunction such as dynamin should be developed. Many markers for synaptic function exist, some of which may lend themselves to detection by radiotracer. The proteins involved in the phenomenon of "silent" synapses could produce additional markers. Silent synapses consume energy but don't signal; they should be investigated. The existing imaging marker of synaptic function, fluoro-deoxyglucose (FDG), is too general and its resolution too low.

There needs to be more emphasis on finding out what happens in individual spines. Their stability/turnover is poorly understood in mammals and is likely to vary regionally and with experience. At the same time, antibody treatments that straighten out dystrophic neurites in mice suggest great plasticity even in the diseased brain. This results needs to be integrated with technology looking at individual spines and seeing how they respond.

## **Imaging**

Participants agreed that the field must broaden its search for non-invasive imaging probes beyond the PET agents FDG and the early-stage PIB. Above all, imaging agents for prions, Lewy bodies, and tau are needed. The latter may be more challenging as tau aggregates and isoforms vary greatly between different tauopathies.

Bill Klunk of the University of Pittsburgh reviewed the current status of the benzothiazole derivative PIB. This agent is being tested in humans at various centers in the US and Europe but is not widely available. It enables rigorous testing of the amyloid hypothesis. It should be used for longitudinal imaging of at-risk populations (e.g. asymptomatic FAD carriers). Such a study would determine the earliest time of amyloid appearance in relation to symptoms and to other markers, including brain imaging and blood/CSF; the effect of immunotherapy could be imaged in a separate cohort. There was consensus that a longitudinal PIB study should include structural MRI, FDG-PET, and functional MRI. The combined pathological-structural-functional data could clarify old questions about regional vulnerability as well as disparities between amyloid-bearing versus functionally affected regions.

Randy Buckner, of Washington University in St. Louis, described an emerging shift in the prevailing view that AD begins in the medial temporal lobe and that disruption there explains the early memory impairment. Recent studies in several labs, including his, Klunk's, and that of Michael Greicius at Stanford University, converge to suggest that posterior cortical regions may be affected early, possibly through disruption of activity-

dependent or even default, resting networks. These areas include the posterior cingulate and retrosplenial cortex; they need further study.

Novel methods are advancing on other imaging fronts relevant to AD and should be imported into the field. For example, in the absence of a crystal structure for presenilin, fluorescent lifetime imaging microscopy (FLIM, which analyzes fluorescent resonance energy transfer) can approximate structural studies of the conformation of active presenilin in live cells with subcellular localization. Ongoing FLIM studies in Brad Hyman's lab at Massachusetts General Hospital in Charlestown analyze PS dimer formation, as well as effects of NSAIDs and of FAD mutations on presenilin conformation and its interaction with APP.

As presented by Bernardo Sabatini of Harvard Medical School, 2-photon calcium imaging and glutamate uncaging methods make it possible to image and analyze the activity of single dendritic spines in cultured brain slices. The approach has not been extensively applied to AD research but, when used in suitable mouse models, could address the effects of PS or APP levels, or FAD mutations, on synaptic structure and function. Finally, molecular imaging of transgenes as developed in the cancer field might prove useful for studies in neurodegeneration, for example PET imaging over time of chaperone activity in AD models.

### **Protein Misfolding**

Participants agreed that protein misfolding occurs with striking similarity among different neurodegenerative diseases, but it remains unclear whether protein misfolding precedes or is secondary to synaptic and axonal dysfunction. It will be important to place protein misfolding and proteasomal/lysosomal degradation into the cascade of AD pathology. On the basic research side, Susan Lindquist, Whitehead Institute, Cambridge, discussed surprising data suggesting that prion conformation of some yeast proteins changes the protein's function and the cell's metabolism, but is not inherently toxic. Indeed, prion formation per se is advantageous for yeast. This raises the question of whether some aggregation-prone proteins have an alternative, self-perpetuating conformation that serves an unknown physiological role, perhaps in synaptic function. One example is shown in yeast/aplysia. Given the strong evolutionary conservation in the way these proteins behave, it is worth examining this question in higher organisms. Participants discussed the concept of natively unfolded proteins, which include tau,  $\alpha$ -synuclein, and A $\beta$ .

Why are aggregates toxic, and which ones are toxic? There was consensus that large aggregates form to protect the cell from more toxic, smaller ones. Large fibrillar aggregates are almost crystalline in their degree of organization. They bury within them the noxious side chains and species that stick out from intermediate species in an almost combinatorial array of different surface groups that have not evolved to be exposed. The slew of oligomeric species is truly different from fibrillar forms in that they are more exposed, less stable, and easier to degrade. Some intermediate species are inherently

toxic. Plaques later cause a secondary, different toxicity, for example by attracting and distorting neurites.

Christopher Dobson of University of Cambridge, UK, suggested that oligomer toxicity has evolved as a way of removing a cell burdened by intermediate species before large amounts can accumulate and infect other cells. Death of an oligomer-laden cell might be a protective response, analogous to death of virus-infected cells. The toxic mechanism of A $\beta$  oligomers in neurons must be determined.

The role of chaperones deserves further study. These proteins may act in opposing ways in neurodegenerative diseases versus cancer, whereby increasing the chaperone balance might protect against the former but predispose to the latter. This is a caveat against drug development based on chaperones. The only risk factor for AD, ApoE, is a chaperone that influences the dynamics of A $\beta$  deposition versus removal. Its mechanism needs more study.

A useful tool for the study of chaperone biology and mechanisms would be a catalog of chaperones in other species, especially in extreme organisms that have evolved chaperones capable of stabilizing proteins in extreme temperatures, osmotic stress, etc.

Is protein misfolding the primary event in AD? Are tau and amyloid changes visible examples of a broader process? How to test these questions?

This research direction arises from work showing that protein folding is a stochastic process with a given error rate. A small amount of misfolding is even necessary so that proteins can be degraded and displayed to induce immune tolerance against self. Under normal conditions misfolded proteins are swept away by degradation systems, preventing aggregation. When this system starts to fail, damage first appears in the most vulnerable cells. A person can have a kilogram of lysozyme in body and still be alive, while a gram of protein aggregated in brain is lethal. Why? This area is wide open for discovery.

### **List of Final Recommendations**

1. Make better mouse models. Create strains with only subtle overexpression under endogenous promoter and authentic spatiotemporal regulation, such as YAC. Recreate humanized APP rat unavailable from Cephalon Inc.
2. Develop an arsenal of imaging markers for pathologies other than amyloid, probes for tau,  $\alpha$ -synuclein. Develop imaging probes that report on functional state of synapse, not just structure of synapse.
3. Test Goldstein's hypothesis. Develop probes for imaging axonal flow in animal models and humans. Study peripheral nervous system defects in axonal transport, search for peripheral markers of that.

4. Develop small-molecule probes that cross BBB and identify particular protein aggregates in brain non-invasively. Does binding predict disease?
5. Determine normal function of APP, PS. Study signaling role of A $\beta$  as lead toward toxicity mechanism.
6. With cell biologists: Elucidate role of APP, A $\beta$ , and oligomers in synaptogenesis and synapse function. Elucidate molecular mechanism of synapse loss and look for synaptic defects directly caused by APP/PS mutations.
7. Better cell biology. Develop models of neurons in 3D matrices as more realistic setting to study A $\beta$  toxicity.
8. Understand process of A $\beta$  aggregation into oligomers. Expand work on good cellular systems for that, such as Lindquist's.
9. Next generation of basic scientists: Have each PI bring a postdoc to workshop.
10. Develop better cognitive assessments of early AD.
11. Develop teams of statisticians and geneticists to follow disease in clinic.
12. Build framework for computational model of the disease process. Support computational modeling of small-molecule oligomer inhibitors.
13. Establish centenarian gene bank to look for protective APP/PS SNPs in sharp old old. In parallel, run unbiased screen for genetic/expression differences in them.
14. Better exploration of interaction between astrocytes and neurons. Astrocyte populations are highly diverse. Field is still at descriptive stage; move beyond that.
15. Run FISH on several hundred sporadic AD cases to look for APP duplication in peripheral cells, like the  $\alpha$ -synuclein triplication causing PD, or Down's.
16. Run massive gene expression and proteomic screens to ascertain whether there are other players that we don't know about yet.
17. Organize quantitative information so it is widely available. Find data sharing mechanisms, such as the [fMRI Data Center](#).
18. Put knowledge pieces together by establishing online AD pathway model. Create an online matrix of mouse, yeast, fly, worm, cell-based model systems.
19. Facilitate establishment of, and access to, DNA collections for genetics studies to identify additional players.

20. Bring structural biologists to the field.