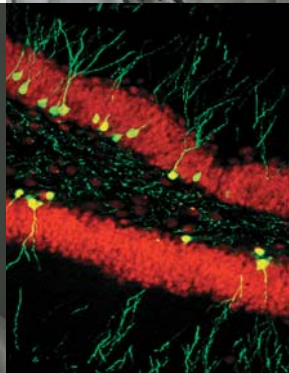
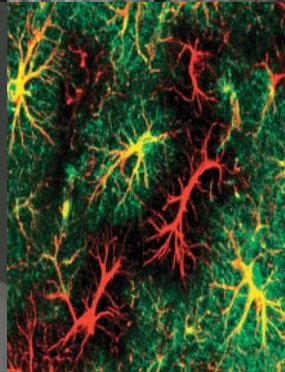
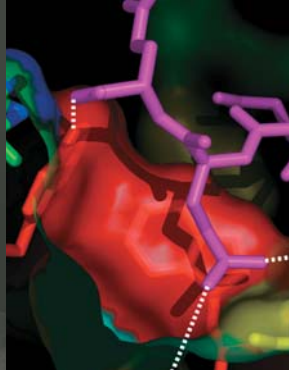
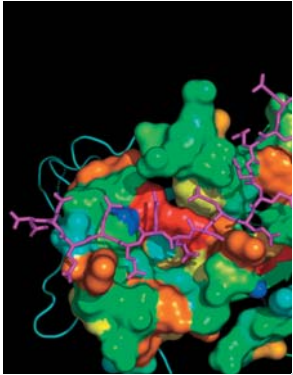


Gladstone Institute of Neurological Disease

Director's Report

Lennart Mucke, MD



Left to right
Katerina Akassoglou
Steven Finkbeiner
Li Gan
Fen-Biao Gao
Yadong Huang
Anatol Kreitzer
Robert W. Mahley
Paul J. Muchowski
Lennart Mucke
Jorge J. Palop
Karl H. Weisgraber

Last year was the busiest year I have experienced during my tenure at Gladstone. Fortunately, it was also among our most successful years in terms of both scientific advances and overall development of the Gladstone Institute of Neurological Disease (GIND). Like the other Gladstone Institutes, the GIND uses highly collaborative, interdisciplinary team approaches to tackle major unresolved biomedical problems.

This includes a drug development program for Alzheimer's disease (AD) and related conditions, in which Drs. Yadong Huang, Robert Mahley, and Karl Weisgraber collaborate with Merck scientists to develop drugs aimed at apolipoprotein (apo) E4, the most important genetic risk factor for AD. Three apoE4-related targets are being pursued in this program and major progress was made last year in the identification of small molecules to correct the disease-causing conformation of apoE4 and in the characterization of an enzyme that generates neurotoxic apoE fragments in neurons. In related studies, Dr. Weisgraber and his team advanced our understanding of the three-dimensional structure of lipid-bound apoE, which could assist in the design of drugs aimed at apoE4. His group further demonstrated that apoE4 impairs not only neurons, but also astrocytes, brain cells that support neuronal functions, adding insult to injury.

The Taube-Koret Center for Huntington's Disease Research is another example of a concerted effort by GIND investigators to attack a devastating neurological disease. Drs. Steven Finkbeiner and Paul Muchowski, who have joined forces in this endeavor, are already ahead of the milestones they defined toward the iden-

tification of better treatments for Huntington's disease (HD) when the center was established not even a year ago. Both of these programs are described more fully in the section on Gladstone's Center for Translational Research.

Another trailblazing effort to develop better treatments for neurodegenerative disorders is the Consortium for Frontotemporal Dementia Research, in which GIND investigators Drs. Li Gan,

“WE WILL CONTINUE TO ADVANCE OUR UNDERSTANDING OF THE NERVOUS SYSTEM AND CONTRIBUTE EVER MORE ACTIVELY TO THE DEVELOPMENT OF BETTER TREATMENTS FOR MAJOR NEUROLOGICAL DISEASES.”

—LENNART MUCKE

Fen-Biao Gao, and Finkbeiner and GICD investigator Dr. Robert Farese collaborate seamlessly with scientists at UCSF and at other centers of excellence across the U.S. GIND investigators played lead roles in two major advances made by this consortium last year: (1) the identification of drugs that can increase the expression of a protein called progranulin, deficiency of which is responsible for a proportion of cases with frontotemporal dementia (FTD), and (2) the establishment

of induced pluripotent stem (iPS) cells from patients with FTD. More information about iPS cells can be found in the GICD section.

There has also been a great deal of progress in individual research laboratories. Two major studies focused on the development of newborn neurons in the context of AD. Stimulating the growth of new neurons to replace those lost in AD is an intriguing therapeutic possibility that has recently moved into the limelight of biomedicine through promising developments in stem cell biology. But will the factors that cause AD allow the new neurons to thrive and function normally? Scientists at the GIND discovered that two main causes of AD—amyloid- β ($A\beta$) peptides and apoE4—impair the growth of new neurons born in adult brains. What is more, they identified drug treatments that can normalize the development of these cells even in the presence of $A\beta$ or apoE4.

Although it had long been assumed that neurons cannot be renewed, it is now well established that new neurons are generated throughout the lives of mammals. One brain region in which new neurons are born in adults, the hippocampus, is involved in learning and memory and affected severely by AD. In collaboration with my group, Dr. Gan studied the development of neurons born in the hippocampus of adult mice genetically engineered to produce high levels of human $A\beta$ in the brain. Surprisingly, $A\beta$ initially accelerated the development of newborn neurons but then profoundly impaired their maturation at later stages of development. Notably, we were able to protect the newborn neurons and ensure their normal development with drugs that counteract $A\beta$ -induced abnormalities in neural network activity. It is possible that

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these drugs could support the development of neurons from stem cells even in the hostile environment of the AD brain.

In a complementary study, Dr. Huang and his team used genetically engineered mice to study the effects of different human apoE variants on the maturation of neural progenitor cells, from which new neurons develop in the adult brain. They found that apoE4 also impairs the development of new neurons in the hippocampus and identified drug treatments that could block these detrimental effects. Their findings suggest that apoE4 inhibits the development of newborn neurons by impairing specific signaling pathways and that boosting these pathways might encourage the development of new neurons from stem cells to replace those lost in apoE4 carriers with AD.

Although stem cell therapy for AD is still a long ways off, these studies have identified strategies to overcome major obstacles in the path toward this goal. They clearly demonstrate that drugs can be used to improve the development of newborn neurons in memory centers of the adult brain, even in the presence of toxic factors widely presumed to cause AD.

Parallel studies have focused on other types of neurodegenerative disease. Dr. Gao and his group showed that a mutant gene that causes FTD in humans misregulates the Toll signaling pathway, which is involved in many important biological processes. What is more, they found that serpin 5, a largely uncharacterized serine protease inhibitor, was able to correct this misregulation, opening up potential new avenues for therapeutic intervention. The investigators also demonstrated that inhibiting autophagy, a process involved in

the degradation of a cell's own components, delayed neuronal loss caused by the same FTD-linked gene.

In contrast, Dr. Finkbeiner and his collaborators showed that pharmacological induction of autophagy protected motor neurons in a different neurodegenerative disease called spinal and bulbar muscular atrophy, highlighting the mechanistic heterogeneity of neurodegenerative disorders and the complexity of the autophagy pathway.

Other studies in the laboratories of Drs. Gao and Finkbeiner focused on TDP-43, an evolutionarily conserved RNA-binding protein implicated in the pathogenesis of FTD, amyotrophic lateral sclerosis (ALS), and other neurodegenerative diseases. Dr. Gao's team revealed an essential role for TDP-43 in the structural integrity of neuronal branches called dendrites, supporting the notion that loss of normal TDP-43 function in diseased neurons may compromise neuronal connectivity before neuronal cell loss occurs in FTD and ALS. Dr. Finkbeiner's group discovered that the toxicity of disease-linked mutations in TDP-43 depends on the mislocalization of TDP-43 from the neuronal nucleus into the cytoplasm.

Using atomic force microscopy, Dr. Muchowski and his coworkers demonstrated that abnormal proteins associated with HD adopt multiple conformations in solution that can be readily distinguished by monoclonal antibodies, a finding that has important implications for understanding the structural basis for the toxicity of these proteins and the development of intrabody-based therapeutics for HD and related conditions.

Many patients with Parkinson's disease (PD) already benefit from the implantation of stimulating electrodes

into motor control centers of the brain, but this approach is still rather crude. More sophisticated approaches are needed to reestablish the fine balance between movement-promoting and -inhibiting circuits that diseases such as PD and HD destroy. Promising advances have been made in this regard by Dr. Anatol Kreitzer through work carried out in our Keck Program for Striatal Physiology and Pathophysiology. Expressing ion channels that can be activated by light in specific groups of neurons in motor control centers and implanting thin fiber optics into the brain in mouse models, Dr. Kreitzer and his group have begun to modulate the activity of neuronal circuits that are affected in patients with HD or PD. Using this system, they can now define the network activity patterns that block or facilitate movements, which should provide useful guidance in the development of better treatments for these disabling conditions.

To further solidify our efforts in AD research, we carried out a rigorous national search for an outstanding investigator with a strong focus on the development of better treatments for this devastating disorder. The joint GIND/UCSF search committee included Drs. Katerina Akassoglou, Ying-Hui Fu, Jennifer Lavail, Robert Messing, Bruce Miller, Paul Muchowski, Brian Shoichet, and myself. Dr. Gan, then a staff research investigator at the GIND, was unanimously identified as the top candidate among a competitive group of applicants. Her research focuses on harnessing A β -degrading enzymes to rid the brain of poisonous A β aggregates, inhibiting detrimental inflammatory activities, and the therapeutic modulation of sirtuins, a fascinating group of

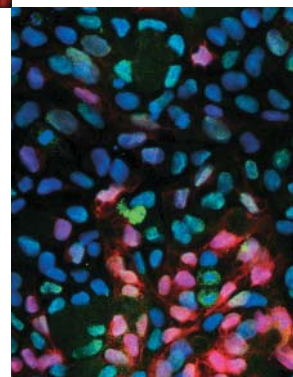
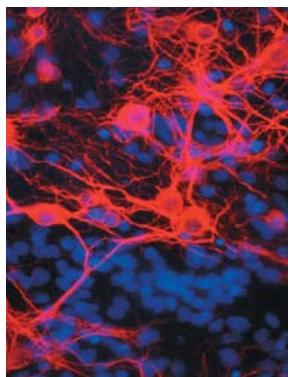
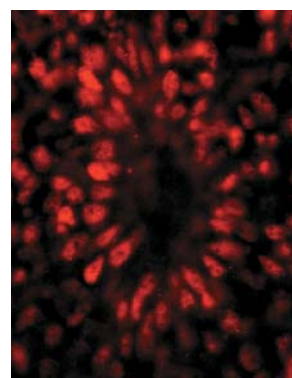
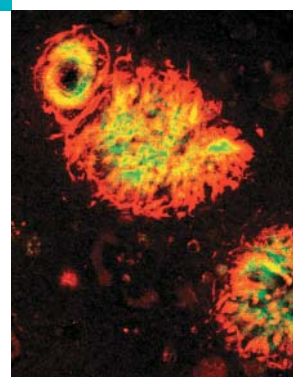
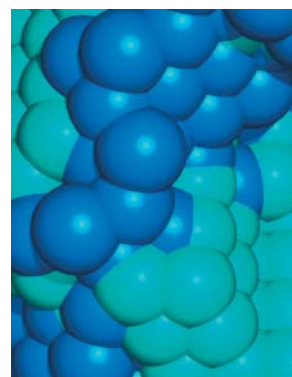
regulatory proteins that play key roles in normal aging and aging-associated diseases. I am delighted that Dr. Gan joined our faculty in September as an assistant investigator and I thank the search committee for their outstanding efforts. I am also indebted to the L.K. Whittier Foundation, the Stephen Bechtel Fund, and Mr. A.W. Clausen, who made this recruitment possible. Dr. Gan holds joint appointments as assistant professor in the Department of Neurology and the Neuroscience and the Biomedical Sciences Graduate Training Programs at UCSF.

As in previous years, GIND scientists promoted collaborations and the exchange of ideas among scientists on a broad scale by organizing symposia. Among them was a behavioral neuroscience symposium, which brought together leading researchers and a capacity crowd of attendees to Gladstone's Mahley auditorium. Organized by Dr. Nino Devidze, who directs the Stephen D. Bechtel Jr. Neurobehavioral Assessment Core at the GIND, the program focused on using animal models to study a range of neurological diseases, including AD, HD, PD, autism, and others. The symposium provided an excellent platform for a fruitful interdisciplinary discussion of the advantages and limitations of using animal models to study these conditions.

Another symposium that triggered lots of positive feedback from the scientific community was co-organized by Dr. Jeffrey Noebels (Baylor College) and myself at the annual meeting of the Society for Neuroscience in Chicago. Entitled "Alzheimer's Disease and Epilepsy: Converging Mechanisms and Therapeutic Opportunities," the symposium highlighted recent findings sug-

gesting an intriguing overlap between AD and epilepsy that might present novel entry points for therapeutic interventions. Dr. Jorge Palop, who was promoted to staff research investigator at the GIND last year, played a key role in connecting these traditionally separate disciplines. His most recent findings suggest that A β elicits aberrant excitatory network activity by impairing specific ion channels on select groups of inhibitory interneurons in the cortex.

I could not be happier about the progress the institute has made during the past year and would like to thank our investigators, trainees, research staff, and administrators, as well as the supporters of our mission, for making these advances possible. Inspired by this year's progress and J. David Gladstone's vision of a healthier future for humankind, we will continue to advance our understanding of the nervous system and contribute ever more actively to the development of better treatments for major neurological diseases.



Katerina Akassoglou, PHD

Fibrinogen, Inflammation, and Neurodegeneration



RESEARCH IN MY LABORATORY focuses on the molecular and cellular mechanisms that are dictated by the extracellular environment after vascular damage and regulate degenerative and repair processes upon injury or disease. We integrate animal modeling, in vivo two-photon microscopy, tissue culture, and biochemical techniques to address the biological complexity of disease and repair mechanisms.

Fibrinogen, a critical component of blood coagulation and inflammation, crosses a damaged blood-brain barrier and accumulates as fibrin deposits at sites of injury. We showed that fibrinogen participates in both the inflammatory response and tissue remodeling/repair in the nervous system and utilizes receptors of neural cells to exert deleterious effects. Understanding the receptor interactions that are responsible for these effects is essential for identifying pharmacologic strategies to block fibrin's deleterious actions in the nervous system without increasing the risk of bleeding or thrombotic events. We also showed that fibrin deposition, scar formation, and tissue repair are under neuronal control. Further, the neurotrophin receptor p75NTR inhibits fibrin degradation at sites of injury by regulating cAMP and promotes cell differentiation during tissue repair in a neurotrophin-independent manner.

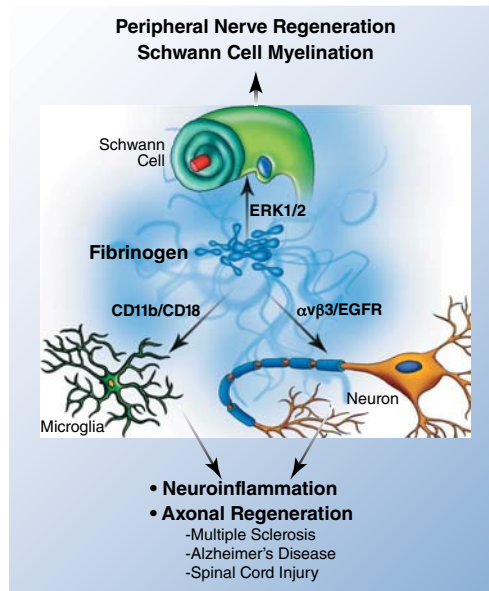
We also solved a difficult technical problem of imaging the neurovascular interface in living mice. Multiphoton microscopy allows direct observation of cells and their behavior in transgenic mice expressing fluorescent proteins driven by tissue-specific promoters in vivo. This approach revolutionized our thinking about synaptic activity, glial

cell motility, and other normal brain functions. We developed a novel method that overcomes the movement artifacts generated by the breathing and heart-beat of the living mouse, enabling us to stably and repetitively image the neu-

rovascular interface in the spinal cord. This approach will allow us to answer fundamental questions related to disease etiology and pathogenesis and identify mechanisms of cell-cell interactions at the neurovascular interface.

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- Sara Gonias
- Matthew Helmrick
- Natacha Le Moan
- Alex Loucks
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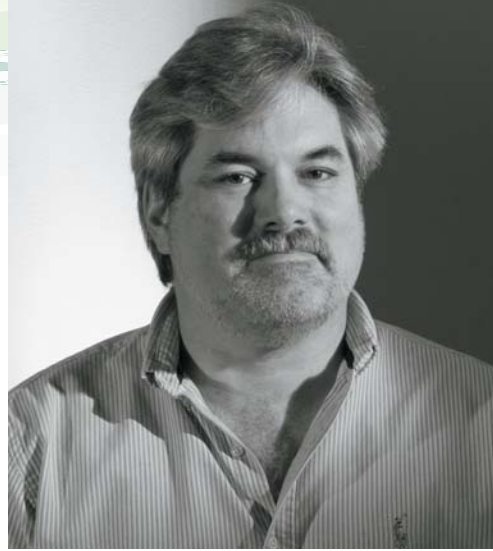
Fibrinogen-induced signal transduction in the nervous system. Fibrinogen elicits diverse biologic responses by inducing distinct signal transduction pathways in neural cells. Fibrinogen induces ERK 1/2 phosphorylation in Schwann cells and inhibits remyelination, activates the CD11b/CD18 integrin receptor in microglia, induces phagocytosis, and phosphorylates the EGF receptor in neurons, causing inhibition and neurite outgrowth. Fibrinogen signal transduction may modulate inflammatory, neurodegenerative, and repair processes in diseases associated with blood-brain barrier disruption and vascular damage.

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Steven Finkbeiner, MD, PHD

Out of Bounds: Mislocation of TDP-43 Causes Neuronal Degeneration



TDP-43 IS A COMMON NUCLEAR PROTEIN

that binds DNA and RNA, inhibits retroviruses, and helps with RNA splicing and nuclear body formation. It also shuttles mRNA to the cytoplasm. However, in patients with amyotrophic lateral sclerosis and certain forms of frontotemporal dementia, TDP-43 is redistributed from the nucleus to the cytoplasm and forms insoluble TDP-43 aggregates in the nucleus, cytoplasm, or processes.

To investigate how TDP-43 might contribute to neurodegeneration, we used genetic engineering to add a fluorescent tag to normal (wildtype) and mutant TDP-43 in primary rat neurons. We used primary neurons because they retain many features of cells in intact brain, including many “normal” features of TDP-43, such as its predominantly nuclear location. To determine the effects of the mutant protein, we used an automated microscope that can examine hundreds of thousands of neurons individually over several days. We then used sophisticated statistics to follow the fate of each individual neuron and determine its risk of death at any given time.

Mutant TDP-43 was toxic to neurons and was abnormally abundant in the cytoplasm. Although the mutant protein formed aggregates called inclusion bodies, they did not affect the risk of cell death. However, the amount of cytoplasmic TDP-43 was a strong independent predictor of neuronal death. We also showed that targeting wildtype TDP-43 to the cytoplasm recreated the toxicity of mutant TDP-43 and that the toxic effect of the mutant protein could be blunted by preventing its export from the nucleus. Thus, the toxicity of the

mutation seems to depend on cytoplasmic mislocalization of TDP-43.

This model system can be used to understand how mutant TDP-43 causes neurodegeneration and provides a way to search for potential therapies.

RECENT PUBLICATIONS

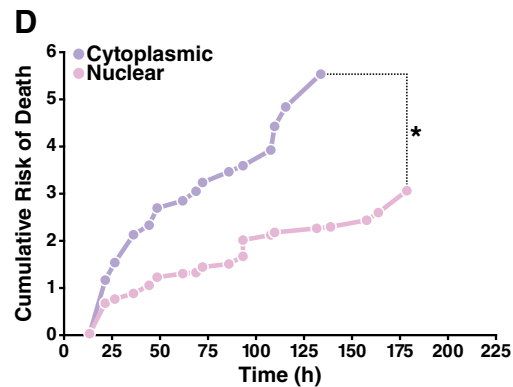
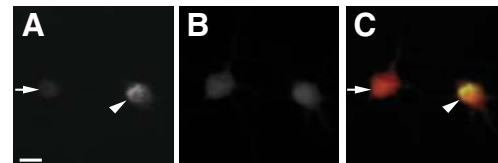
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Cytoplasmic TDP43-EGFP significantly increases the risk of death in transfected neurons. (A–C) Cells displaying purely nuclear (arrow) or both nuclear and cytoplasmic (arrowhead) TDP43-EGFP were identified by fluorescence microscopy. (A) EGFP fluorescence. (B) mCherry fluorescence. (C) Merged image with EGFP fluorescence in green, mCherry fluorescence in red, and overlap in yellow. Scale bar, 10 μ m. (D) Kaplan-Meier survival analysis of neurons with matched expression levels and either nuclear localization of TDP43-EGFP (nuclear, n = 153) or nuclear and cytoplasmic localization (cytoplasmic, n = 258), demonstrating the toxicity of cytoplasmic TDP43-EGFP.

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Li Gan, PHD

Unbalanced Neuronal Activity Impairs Adult Neurogenesis in Alzheimer's Disease Models



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THE REGION OF THE BRAIN called the hippocampal formation is critical for learning and memory and is especially vulnerable to neuronal loss in Alzheimer's disease (AD). It is also one of the few regions in which new neurons are constantly being "born" in adult rodent and human brains. Adult-born neurons arise from neural stem cells, which can develop into several types of brain cells and thus have the potential to replace degenerating neurons in AD. However, the pathogenic and aging microenvironment in AD brains might adversely affect the development of neural stem cells into mature neurons. One aspect of our research focuses on tackling key challenges and exploring the potential of regenerative strategies in AD.

We first assessed the development of neural stem cells in an AD mouse model, which has high levels of amyloid β ($A\beta$), a key pathogen in AD, and develops age-dependent neuropathology and cognitive deficits. Newborn neurons were labeled in vivo with a green fluorescent protein (GFP)-expressing retroviral vector, which only labels cycling progenitor cells. At different times after the neurons were born, we quantitatively assessed their dendrites and spines and their electrophysiological responses. Immature neurons (2–3 weeks old) in AD mice had significantly more spines and stronger responses than those in age-matched mice. However, at maturity at 4–6-weeks, they had shorter dendrites, fewer spines, and weaker responses. Thus, neural stem cells in AD mice developed abnormally quickly at early stages but failed to mature and integrate properly at late stages.

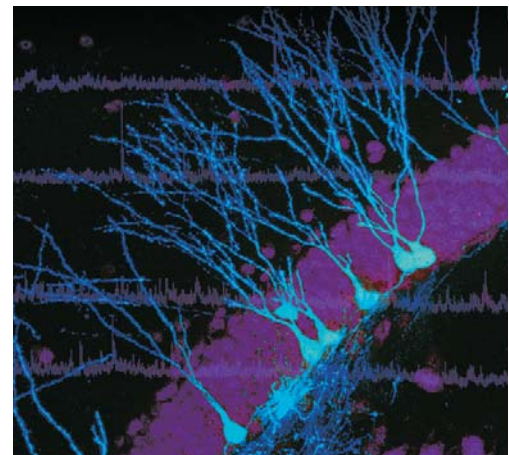
What could cause these biphasic alterations? A key step that converts immature neurons to mature neurons is the switch of γ -aminobutyric acid (GABA) signaling from excitatory to inhibitory. Since AD mice have more GABAergic contacts onto neurons in the dentate gyrus, we hypothesized that excessive GABAergic signaling was responsible for the abnormal development of neural stem cells in AD models. Indeed, inhibition of the GABA_A receptor with its antagonist largely restored the dendritic and spine maturation of these neurons. Glutamatergic signaling also regulates the maturation of neural stem cells in hippocampus, and $A\beta$ -induced depression of glutamatergic transmission requires activation of calcineurin. Inhibiting calcineurin with the small-molecule inhibitor FK506 completely restored the dendritic maturation of adult-born neurons. These results implicate excessive GABAergic signaling and depressed glutamatergic signaling as mechanisms of impaired neurogenesis in AD models. Our findings could also lead to pharmacological strategies to improve the functionality of endogenous, and possibly transplanted, neural stem cells, in the presence of high levels of $A\beta$.

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Adult-born granule cells (blue) in the dentate gyrus of the adult mouse hippocampus. Neu-N was used to label all mature neurons (purple). Representative recordings of miniature inhibitory postsynaptic currents from adult-born granule cells are shown as purple traces.

Fen-Biao Gao, PHD

Understanding Neurodegenerative and Neurodevelopmental Diseases



OUR LABORATORY seeks to understand the pathogenesis of frontotemporal dementia (FTD) and related disorders and to identify therapeutic targets. FTD is a common cause of dementia in patients under 65 years of age and has a variable clinical presentation and genetic basis. Several molecules contribute to its pathogenesis, including CHMP2B, progranulin, and TDP-43.

CHMP2B, the gene encoding the human homolog of the yeast endosomal sorting complex required for transport component Vps2, is mutated in a rare form of autosomal dominant FTD. We found that a small-molecule inhibitor of autophagy—an evolutionarily conserved protein degradation pathway—delays neuronal cell loss caused by the mutant CHMP2B. siRNA knock down of the autophagy genes *atg5* and *atg7* had a similar effect. Thus, under certain neurodegenerative conditions, autophagic stress by excess accumulation of autophagosomes might be detrimental to neuronal survival. If so, partial inhibition of autophagy induction is a potential therapeutic strategy for some forms of FTD.

This year, we established a fly model of FTD3, completed an unbiased genome-wide modifier screen, which identified 29 genetic loci, and cloned four evolutionarily conserved modified genes. One encodes a serine protease inhibitor, indicating involvement of an important signaling pathway in mutant CHMP2B toxicity. These findings will be validated in mouse models of FTD.

We also investigated how mutations in the gene encoding progranulin, a secreted protein of unknown function, cause FTD. Progranulin mutations often lead to a characteristic TDP-43 pathology. TDP-43, an evolutionarily

conserved, largely nuclear RNA-binding protein, was depleted in the nucleus of diseased neurons, suggesting a pathogenic mechanism that involves loss of its normal nuclear function. We also found that *Drosophila* TDP-43 is required for neuronal dendritic integrity. Moreover, human TDP-43 promoted dendritic branching in *Drosophila* neurons, and this function was attenuated by mutations associated with neurodegenerative diseases. Our fly model of TDP-43 biology will be useful for understanding FTD and associated disorders.

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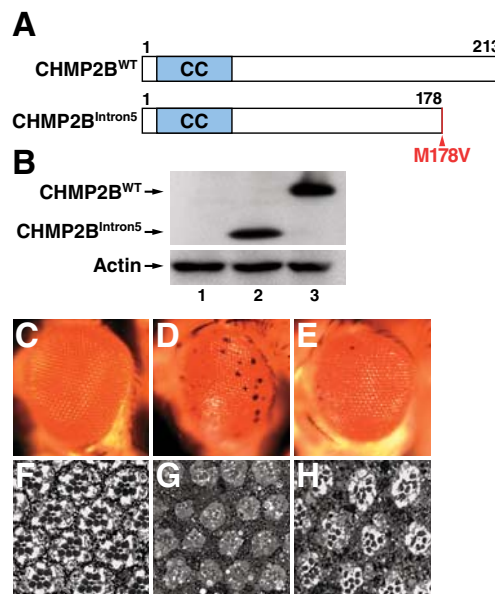
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A fly model of FTD3. (A) Wildtype and FTD3-associated mutant CHMP2B proteins. CC: coiled-coil domain. CHMP2B^{Intron5} lacks the C-terminal 35 amino acids, and methionine 178 is changed to valine. (B) Western blot analysis. Lane 1: *GMR-Gal4*; lane 2: *GMR-Gal4:UAS-CHMP2B^{Intron5}*; lane 3: *GMR-Gal4:UAS-CHMP2B^{WT}*. (C–H) CHMP2B^{Intron5} expression caused severe degeneration in the *Drosophila* eye. (C, F) *GMR-Gal4* flies had normal external morphology (C) and internal retinal structure (F). (D, G) CHMP2B^{Intron5} expression in the eye caused black spots (D) and degeneration of internal structures in 1-day-old flies (G). (E, H) CHMP2B^{WT} expression caused a mild black spot phenotype (E) and had a minor effect on the internal structure in 1-day-old flies (H).

Yadong Huang, MD, PHD

Apolipoprotein E4 Impairs the Growth of New Nerve Cells in Adult Brains



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 Victoria Yoon
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STIMULATING THE GROWTH of new neurons to replace those lost in Alzheimer's disease (AD) is an intriguing therapeutic strategy. But will the risk factors that cause neurodegeneration in AD also hinder the development of new nerve cells? To address this issue, we examined the role of apolipoprotein (apo) E4, the greatest known genetic risk factor for AD, on the differentiation of neural stem/progenitor cells (NSCs) in the hippocampus of adult mice. The hippocampus is one of the first regions of the brain damaged in AD, and memory deficits and disorientation are among the early symptoms. NSCs are stem cells that have begun to commit to a cell fate to become one type of brain cell (e.g., glia, neuron). We used adult mice in which genetic engineering was used to knock out (KO) the gene for mouse apoE or to knock in (KI) the gene for human apoE3 or apoE4. We found that NSCs express apoE and that the development of newborn neurons was less efficient in the apoE-KO and apoE4-KI mice. The apoE-KO mice had more bone morphogenetic protein signaling, which promoted the differentiation of NSCs into glia rather than into neurons in the hippocampus. In apoE4-KI mice, presynaptic GABAergic signaling to developing neurons was reduced. Since GABAergic signaling is critical for neuronal development, this reduction explains apoE4's detrimental effect on neurogenesis. By enhancing GABAergic signaling, we restored normal neurogenesis in the hippocampus of apoE4-KI mice.

These findings suggest that apoE4 inhibits the development of newborn neurons by impairing GABAergic signaling. Boosting this signaling pathway with drugs may be of therapeutic ben-

efit. It might allow us to encourage the development of new neurons from stem cells to replace those lost in apoE4 carriers with AD.

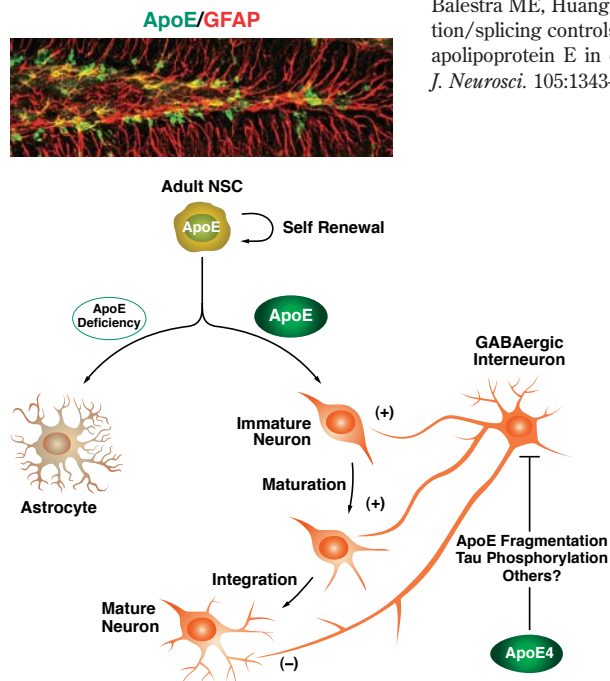
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A working model for the roles of apoE in adult hippocampal neurogenesis. Adult hippocampal NSCs express apoE, which plays an important role in cell-fate determination of NSCs toward neuronal development. ApoE deficiency stimulates astrogenesis and inhibits neurogenesis. ApoE4 decreases hippocampal neurogenesis by impairing presynaptic GABAergic input onto newborn neurons, inhibiting their maturation.

Anatol Kreitzer, PHD

Adaptive and Maladaptive Patterning of Motor Behavior by Basal Ganglia Circuits



MOVEMENT DISORDERS, such as Parkinson's disease (PD) and Huntington's disease (HD), arise from the dysfunction and eventual loss of neurons in the basal ganglia, a region located deep in the core of the brain that is involved in motor planning functions. Although the anatomy and physiology of basal ganglia neurons have been studied extensively over the past several decades, it is still not clear how these millions of neurons act in concert to control movement.

In the brain, all neurons are connected to other neurons, forming so-called neural circuits. Information, in the form of electrical impulses, is transmitted through a variety of such circuits to mediate complex behaviors. Circuits of the basal ganglia are important for transforming information to help us select the appropriate motor programs to perform in a given situation, such as picking up a cup of coffee, turning a door handle, or sitting down in a chair. However, the way in which basal ganglia circuits accomplish this task remains a mystery.

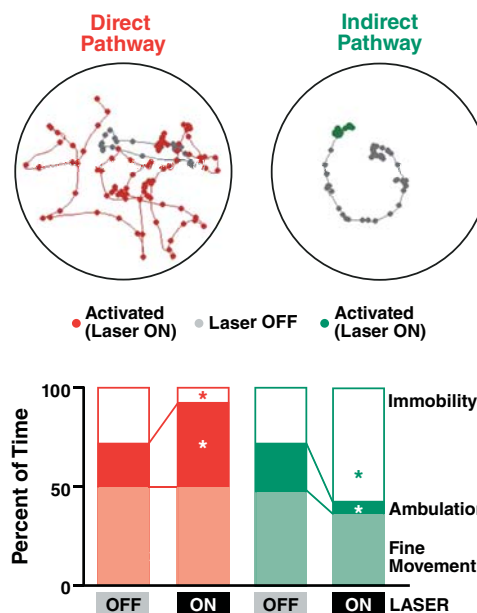
To probe the function of basal ganglia circuits in living animals, we inserted a protein called channelrhodopsin-2 into neurons that form the origin of two major basal ganglia circuits: the direct pathway and the indirect pathway. Channelrhodopsin-2 is a light-sensitive ion channel derived from green algae that allows neurons containing this protein to be activated by blue light. By introducing light into the brain with thin fiber optics, specific neural circuits can be precisely controlled, leaving other nearby neurons and circuits unaffected. Using this technology, we established the causal role of basal ganglia circuits in patterning motor behavior

for the first time. In mice, we found that direct pathway activation leads to hyperactivity, characterized by jerky, uncoordinated movements and increased locomotion—behavior similar to that observed in patients with HD. Indirect pathway activation caused mice to freeze for long periods, similar to behavior in patients with advanced PD. Since we can induce these behaviors by activating basal ganglia neural circuits, the next step will be to inhibit these circuits in mouse models of HD and PD and test whether this can restore motor function. If this is indeed the case, targeting neural activity in basal ganglia circuits may be a promising therapeutic avenue for treating these devastating diseases.

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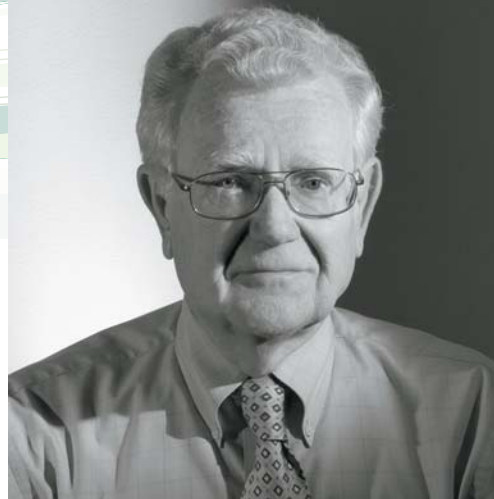
Mouse behavior during activation of direct pathway (left) or indirect pathway (right) basal ganglia circuits. A thin optical fiber was used to deliver blue laser light into the brain. The light activates channelrhodopsin-2 and increases neural circuit activity in the specified pathway. Above, circles delineate the mouse's enclosure. Lines represent the mouse's path, and dots along the path show the mouse's location every 300 msec. Gray lines are before laser illumination; colored lines are during laser illumination. Bottom, summary data showing motor behavior during experimental trials before (left) and after (right) direct pathway activation (red bars) or indirect pathway activation (green bars). Asterisks indicate statistically significant differences for the "laser ON" versus "laser OFF" periods.

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Robert W. Mahley, MD, PHD

Apolipoprotein E4: Structure Determines Function in Neuropathology



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APOLIPOPROTEIN (APO) E is the major known genetic risk factor for Alzheimer's disease and has a potential role in other neurological diseases and traumatic brain injury. ApoE4 can cause neuropathology directly, and it sets the stage for "second hits" to precipitate neuropathology. The neuropathologic effects of apoE4 are mediated by its structural features, such as domain interaction. It is particularly prone to assume a pathological conformation. When stressed or injured, neurons synthesize apoE. ApoE4 uniquely undergoes neuron-specific proteolysis, yielding toxic fragments that disrupt mitochondrial energy balance and cause cell death.

Mitochondrial respiratory complexes were examined in neurons cultured from brain cortices of neuron-specific enolase promoter-driven apoE3 (NSE-apoE3) or apoE4 (NSE-apoE4) transgenic mice. All subunits of mitochondrial respiratory complexes assessed were significantly decreased in NSE-apoE4 neurons compared with NSE-apoE3 neurons. In Neuro-2a cells, levels of mitochondrial respiratory complexes I, IV, and V were decreased by apoE4. Enzymatic activity in complex IV was also decreased, leading to reduction in mitochondrial respiratory capacity. Mutant apoE4 (apoE4-Thr61) lacking domain interaction did not induce mitochondrial dysfunction, indicating that the mitochondrial dysfunction is apoE4-specific and domain interaction-dependent. Likewise, treatment with a small molecule that interferes with apoE4 domain interaction restored complex IV levels in apoE4-expressing cells.

These findings demonstrate that apoE4 induces mitochondrial dysfunction in neuronal cells through its

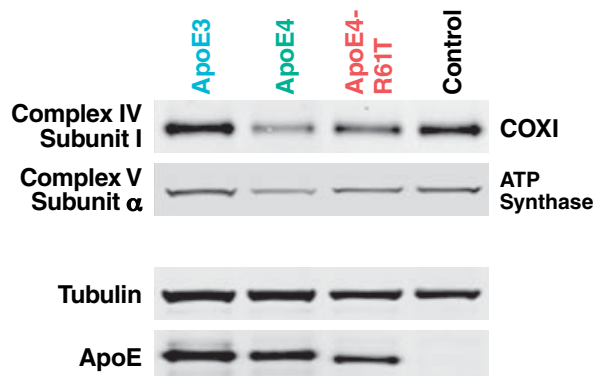
domain interaction. Thus, pharmacological interventions that disrupt this interaction with small molecules might hold therapeutic potential for apoE4-carrying Alzheimer's disease patients.

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ApoE4 expression in Neuro-2a cells results in decreased amounts of mitochondrial cytochrome C oxidase (COXI) and ATP synthase.

Paul J. Muchowski, PhD

Polyglutamine in Huntingtin Dances the Conformational Cha-Cha-Cha

AT LEAST NINE HUMAN PROTEINS with a long repeating stretch of a single amino acid called glutamine cause inherited neurodegenerative diseases. Huntington's disease (HD) is caused by a genetic mutation that leads to the expansion of a polyglutamine (polyQ) repeat in the protein huntingtin (htt). The pathogenic mechanism of HD is a mystery. One clue to solving this mystery is that the polyQ expansion in mutant htt causes the protein to misfold, aggregate, and form insoluble deposits in the neurons of HD patients. Similar deposits of aggregated proteins are also found in many other neurodegenerative diseases.

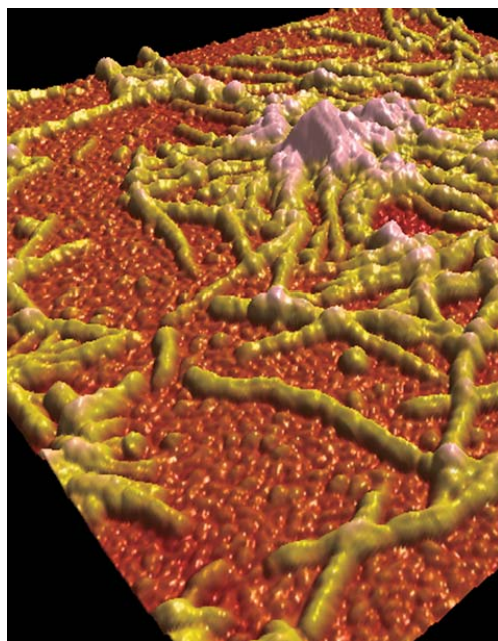
We hypothesized that small aggregated forms of htt called oligomers interact abnormally with other neuronal proteins in a manner that impairs their normal functions. However, the mutant htt forms oligomers with a wide variety of structures. A major difficulty in determining which structures might be pathogenic is the lack of good tools to track these abnormal assemblies in HD brains.

Monoclonal antibodies are attractive tools to measure different misfolded conformations of htt in neurons and to potentially determine their pathogenic significance in HD. Computational approaches suggested that polyQ repeats assemble into a large number of misfolded conformations—analogueous to a metal chain with flexible links that allow it to adopt many different shapes. We decided to see how a panel of monoclonal antibodies that detect the polyQ repeat in htt affect its aggregation. If polyQ repeats form different conformations, monoclonal antibodies should be able to recognize these structures and would have differential effects on htt aggregation. Using atomic force microscopy (AFM), a tech-

nique that allows one to image mutant htt aggregates at nanometer resolution, we found that monoclonal antibodies had widely varying effects on htt aggregation. Antibody 3B5H10, developed by our collaborator Steven Finkbeiner, even dissolved pre-formed aggregates.

Remarkably, the polyQ repeat in htt appears to freely move through different conformations. Our findings were

confirmed by a study showing at atomic resolution that polyQ repeats in htt stably adopts multiple discrete conformations. These results have implications both for the structural basis for mutant htt toxicity and for therapies, as antibodies delivered by gene therapy have shown promise in mouse models of HD and are being developed for clinical tests in HD patients.



Atomic force microscopy image of mutant htt aggregates. This technique was used to show that monoclonal antibodies recognize discrete conformations of polyQ in mutant htt that might mediate the formation of different aggregate species.

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Lennart Mucke, MD

A β and Tau: Partners in Crime



ALZHEIMER'S DISEASE (AD) is devastating, costly, incurable, and increasing in prevalence in aging populations around the world. Thus, there is an urgent need to deepen our understanding of this common neurodegenerative disorder and to develop better strategies to treat and prevent it. Amyloid- β (A β) peptides are widely thought to cause AD, and many new AD treatments now in clinical trials aim to lower the production of A β or to enhance its removal. However, the efficacy and long-term safety of these treatments remain unknown. Therefore, it is important to search for alternative or complementary therapeutic approaches, including strategies to make the brain more resistant to A β 's disease-causing effects.

Using mouse models of AD, we discovered that even a partial (50%) reduction of tau levels can prevent A β from causing cognitive deficits and related neuronal abnormalities. Tau reduction also made mice more resistant to chemically induced seizures, suggesting that it might be beneficial in a variety of neurological diseases associated with abnormal neuronal excitation. If we knew how tau reduction protects the brain so effectively against A β and other excitotoxins, it might be possible to simulate its protective effects pharmacologically without having to reduce tau itself.

During the last year, we made major progress in unraveling the mechanisms underlying the beneficial effects of tau reduction. Specifically, we discovered that tau reduction prevents A β -induced abnormalities in synaptic plasticity (or long-term potentiation), which may underlie AD-related deficits in learning and memory. Tau reduction also blocked aberrant excitatory neuronal activity

resulting from disinhibition, which is increasingly recognized as an important cause of neural network instability in AD. Furthermore, tau reduction counteracted A β -induced alterations in the intracellular distribution of molecules that affect neuronal excitability. These and follow-on experiments currently under way could pave the way toward the development of better treatments for AD and other devastating neurological disorders.

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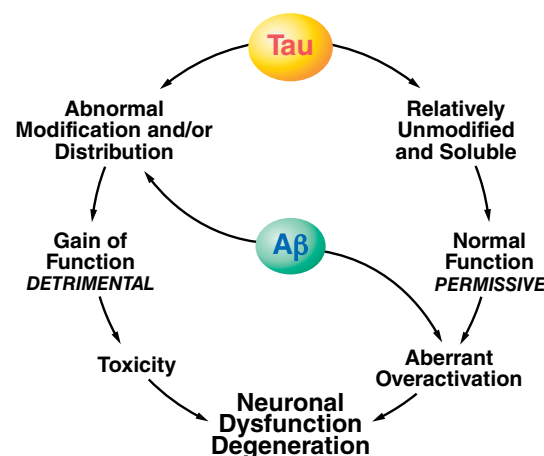
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Potential ways in which A β and tau may conspire to impair neuronal functions in AD. The hypotheses depicted on the left and right are not mutually exclusive, but the one we posed on the right is more novel and has yet to be fully explored.

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- Alex Chung
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 - Dena Dubal
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 - Julie Harris
 - Kaitlyn Ho
 - Daniel Kim
 - Akihiko Koyama
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 - Meaghan Morris
 - Lennart Mucke
 - Anna Orr
 - Pascal Sanchez
 - Hilda Solanoy
 - Elsa Suberbielle
 - Myo Thwin
 - Sandy Toh
 - Keith Vossel
 - Xin Wang
 - Gui-Qiu Yu

Jorge J. Palop, PHD

Connecting Synapses with Circuits: Synaptic Depression and Aberrant Excitatory Network Activity in Alzheimer's Disease



ALZHEIMER'S DISEASE (AD) has been traditionally viewed as a progressive dismantling of glutamatergic synapses, circuits, and neurons associated with a build-up of amyloid β ($A\beta$) and tangle pathology. Experimental mouse and cell-culture models have supported this view for decades. In vitro and in vivo studies from many laboratories showed that high levels of $A\beta$ effectively suppress synaptic transmission strength and plasticity at specific glutamatergic synapses. However, the net effects on overall neuronal activity at the network level were unexplored.

Recently, we found that transgenic models of AD with high levels of $A\beta$ have generalized epileptiform activity and nonconvulsive seizures involving cortical and hippocampal networks. These unexpected results indicate that synaptic depression and aberrant network activity coexist in AD and suggest that they might be mechanistically related. Importantly, patients with AD, particularly those with early-onset AD or familial AD, have an increased incidence of convulsive seizures. Such aberrant neuronal activity has been widely interpreted as a secondary process resulting from neurodegeneration. However, our findings challenge this notion, raising the possibility that seizure activity in AD may represent a primary upstream mechanism triggered by high levels of $A\beta$.

My laboratory focuses on determining the molecular mechanisms of $A\beta$ -induced epileptogenesis and the downstream consequences of aberrant neuronal activity on cognition. Our working model of $A\beta$ -induced cognitive dysfunction proposes that high levels of $A\beta$ lead to aberrant neuronal activity

and compensatory inhibitory responses involving learning and memory circuits. At the molecular level, we are actively exploring whether reduced levels of voltage-gated sodium channels (VGSC) on fast-spiking GABAergic interneurons lead to $A\beta$ -induced epileptiform activity. Confirmation of a causal relationship between $A\beta$ -induced aberrant excitatory neuronal activity, VGSC alterations, hippocampal inhibitory remodeling, and

cognitive decline would provide important insights into the pathogenesis of AD and could open new therapeutic avenues.

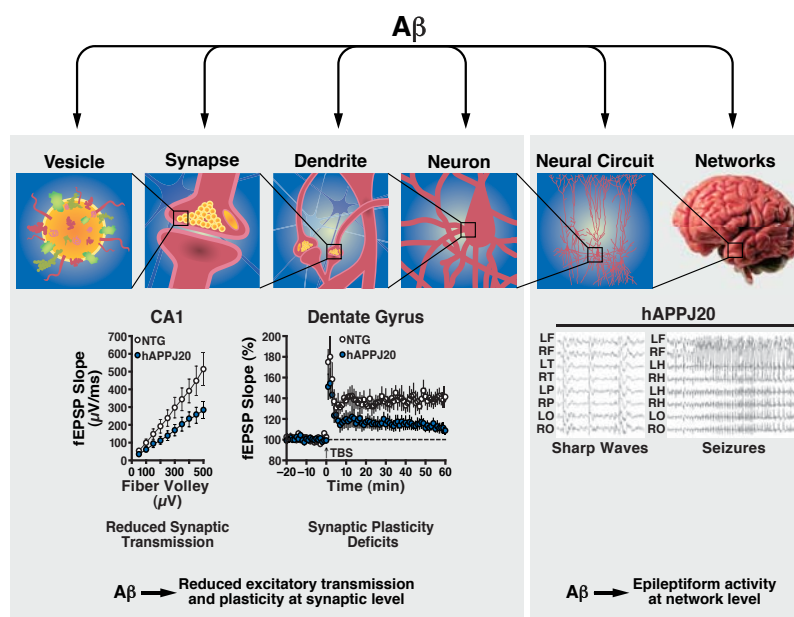
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Palop Laboratory Members

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Laure Verret



$A\beta$ affects neuronal activity at multiple levels. High levels of $A\beta$ depress excitatory synaptic transmission and impair synaptic plasticity at the synaptic level (left), but elicit epileptiform activity and seizures at the network level (right). Although it is unlikely that $A\beta$ exerts independent effects at each level, the extent to which these alterations are mechanistically related is unknown.

Karl H. Weisgraber, PHD

Astrocytes May Contribute to Apolipoprotein E4-mediated Neurodegeneration



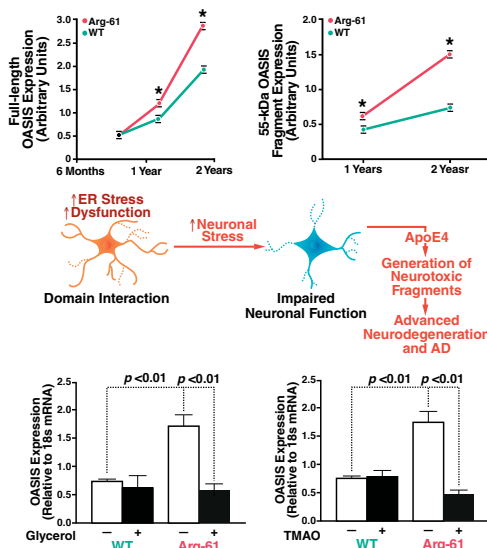
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Belinda Cabriga
Jennifer Hsu
Yvonne Newhouse
Earl Rutenber
Karl Weisgraber
Ning Zhong

APOLIPOPROTEIN (APO) E4, the major known genetic risk factor for Alzheimer's disease (AD), differs structurally from the other apoE isoforms in two ways. First, a salt bridge between Arg-61 in the N-terminal domain and Glu-255 in the C-terminal domain causes the apoE4 domains to interact. Second, apoE4 is less stable and displays a molten globule state. Because structure is often closely related to function, we sought to determine the relative contribution of these properties to neurodegeneration. We generated a mouse model specific for domain interaction. In these Arg-61 apoE mice, apoE is produced primarily, if not exclusively, by astrocytes and other glial cells.

The Arg-61 apoE mice have synaptic, functional, and mild cognitive deficits. Arg-61 apoE does not accumulate in astrocytes, indicating that it is recognized as abnormally folded and targeted for degradation. This observation raised the possibility of an unfolded protein response (UPR). We determined that all three UPR pathways were activated, suggesting that Arg-61 apoE astrocytes might be dysfunctional. Consistent with this hypothesis, the level of the astrocyte glutamate transporter 1 in the hippocampus was reduced in Arg-61 apoE mice, raising the possibility of excitotoxicity. Also, glucose uptake by Arg-61 apoE astrocytes was impaired, and conditioned medium from Arg-61 astrocytes was less effective in promoting neurite outgrowth.

Thus, apoE4 domain interaction may cause astroglial dysfunction that contributes to neuronal deficits in Arg-61 apoE mice. We hypothesize domain interaction impairs the ability of astrocytes to support neuronal function, although the cells remain marginally effective in the



Working model of the association of astrocyte ER stress with neurodegeneration. Domain interaction in apoE4 causes the organism to be more susceptible to stress-related damage with age. Chemical chaperones help apoE4 to assume a more apoE3-like structure and reduce stress and dysfunction.

absence of brain stress. With added brain stress—from aging, ischemia, oxidative stress, head trauma, or A β toxicity—astrocytes express additional apoE for neuronal repair. ApoE4, exacerbates endoplasmic reticulum (ER) stress, further compromising astrocyte support. Neurons begin apoE expression as a protective mechanism, resulting in neurotoxic apoE4 fragments, as shown by Yadong Huang.

To test this hypothesis, we examined the effect of age on ER stress in mice. We used the astrocyte-specific UPR marker,

OASIS, and its transcriptionally active fragment, which activates downstream UPR target genes. Both OASIS and its fragment indicated increased stress with age, consistent with the age-dependent loss of the presynaptic protein marker synaptophysin. In addition, two chemical chaperones, glycerol and trimethylamine oxide, were tested to determine if they could reduce ER stress. Both agents reduced OASIS levels in cultured astrocytes.

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