While research conducted today may take years to influence clinical practice and the lives of people living with Alzheimer’s disease, funding for that research has an immediate effect on the scientists whose work is supported. Receiving Association funding can play a critical role in advancing scientists’ research and, for some, put them on a path toward becoming leaders in Alzheimer and dementia research. In their own words, investigators Drs. Chad Dickey, Deborah Barnes, Orly Lazarov, Mary Mittelman, Henrik Zetterberg, Robia Pautler and Giulio Tagliatela describe their research and what receiving Association funding has meant to their careers.
Without support from the Alzheimer’s Association, the following work may never have been done. Moreover, it is quite possible that I might have left science or changed fields simply due to the extremely challenging funding environment that currently exists in science as a result of the economic crisis we face in the United States.

With the $100,000 provided by the Alzheimer’s Association, we implemented a novel screening technique that not only identifies drugs that may be new treatments for Alzheimer’s disease, but also sheds light on new mechanisms for identifying better drug targets in the future. In particular, we have focused on using chemicals to promote recycling and clearance of the tau protein, which accumulates in abnormal amounts in the brains of those with Alzheimer’s.

We found that over-expression of mutant human tau impairs the turnover of otherwise normal tau in the mouse brain. We also found that young mice are capable of clearing tau while older animals are not. In addition, we found that heat shock proteins, which are essential for preserving tau in the brain, were present in low levels in young mice relative to old mice. This could indicate that heat shock proteins are contributing to tau pathology.

Using drugs from our screen that inhibit heat shock proteins, we found that if other proteins are accumulating in the brain at the same time as tau, there might be a cellular ‘log-jam’ at the protein clearance system, leading to aggregation of tau.

Other drugs identified from our screen were then tested in mice. Twenty-four hours after injection we found that methylene blue (MB) and its derivative azure C (AC) could effectively lower tau levels in the brain. We are currently analyzing data from a long-term study with MB that show that memory function is improved in mice treated with MB compared with untreated mice. We also saw a reduction in tau levels in mice treated with MB.

We are combining some of these therapies based on their mechanism of action to improve efficacy. Interestingly, MB was recently proposed to inhibit tau protein aggregation, and this function, coupled with its low toxicity profile, propelled it into clinical trials for Alzheimer’s, where it has been met with guarded enthusiasm.

Through our screen and subsequent work, we have identified that MB reduces tau levels by inhibiting a heat shock protein called Hsp70. In collaboration with the Gestwicki Laboratory at the University of Michigan, we have found that Hsp70 activators significantly increased tau levels. Collectively, these data demonstrated that the breakdown of tau is rapidly facilitated when Hsp70 is inhibited by MB. It also suggests that Hsp70 activity prevents the degradation of tau, underscoring its value as a therapeutic target for Alzheimer’s.

Thus, while the use of MB in the clinic is entering Phase III clinical trials for the treatment of Alzheimer’s disease, its role as an aggregation inhibitor may be superfluous to its more important function of inhibiting Hsp70 activity. By identifying the primary target of MB as Hsp70, the development of more potent Hsp70 inhibitors with superior ability to cross the blood-brain barrier can begin. This discovery could prove to be a critical cornerstone for other
neurodegenerative diseases and make Hsp70 inhibition a viable therapeutic approach. By identifying Hsp70 as a major contributor to tau degradation, we have a more complete understanding of how Alzheimer’s disease starts.

The faith that the Alzheimer’s Association places in junior investigators by providing them with a possible funding source has been critical for progress in the field. By providing an influx of new ideas and scientists in the Alzheimer community, therapies that likely would never have been considered may get to the clinic for Alzheimer’s patients.

One of the things that the general public is likely unaware of is the expense of basic research. This comes in the form of supplies from biotech companies, housing costs for animals and, most important, salary support for the scientists and students involved in the research efforts.

As the recipient of a New Investigator Research Grant (NIRG), I can personally attest that without the grant, I would not have had the same success that I had early on in my training. I received the NIRG one year after I was promoted from a post-doctoral position to an assistant professor at the Mayo Clinic. The NIRG gave me the autonomy I needed to pursue my own ideas, which then led to subsequent funding from the National Institutes of Health. The NIRG funds, coupled with the additional resources I secured as a result, allowed me to take a promotion at the University of South Florida and eventually be retained into a tenure-track position where I received start-up funds from the University and laboratory space in the Byrd Alzheimer’s Institute.

To be quite frank, I likely would have changed to the cancer biology field had it not been for the NIRG I received from the Alzheimer’s Association simply because the funds available for cancer research are so much greater than for Alzheimer’s. Providing these grants to new investigators at a stage when we are just establishing ourselves in the field gives us just enough resources to persevere in our work on Alzheimer’s despite the poor economic climate. These funds provide the foundation for future clinical successes in Alzheimer’s, as evidenced, in small part, by the progress we have made over the past three years toward our ultimate goal: to identify drugs that modify the biological processes leading to the accumulation of tau and perhaps improve the quality of life for millions of people living with Alzheimer’s.”

— CHAD DICKEY, PH.D.
My research program focuses on identification of factors that may protect against Alzheimer’s disease and other dementias and on evaluation of interventions to enhance cognitive function and delay dementia onset. Several of my prior studies have examined the potential protective effects of mental activity on cognitive function in older adults. In one study, we found that older adults with higher literacy levels had better cognitive function on a wide range of measures. I also was a co-investigator on a pilot randomized controlled trial (RCT) to determine whether a computer-based mental training program could improve cognitive function in older adults with mild cognitive impairment.

In addition, I have conducted several studies of the potential beneficial effects of physical activity on cognitive function in older adults. My colleagues and I performed one of the earliest longitudinal studies in this area in which we found that older women who reported engaging in more physical activity were less likely to experience cognitive decline over 6 to 8 years of follow-up than those who were not as physically active. Because we were concerned about the potential for bias related to self-report of activity levels in this study, we conducted two studies that used more objective physical activity measures, including cardiorespiratory fitness as assessed by peak oxygen consumption during exercise treadmill testing and daytime movement as assessed by an activity sensor worn on the wrist. These produced similar results to those of our earlier studies. Evidence from our work and the work of others led us to publish a review article calling for RCTs to determine whether exercise can prevent dementia in at-risk elders.

Funding from the Alzheimer’s Association combined with a Career Development Award from the National Institutes of Health (NIH) enabled me to launch my first independent RCT. In this trial, we are building on our prior work by comparing the effectiveness of physical and mental activity for enhancing cognitive function in non-demented, inactive older adults who report a recent decline in memory or thinking. This trial, called the Mental Activity and eXercise (MAX) Trial, uses a double-blind, factorial design in which participants are randomly assigned to a mental activity intervention or control group and an exercise intervention or control group. This design will enable us to compare the effectiveness of physical and mental activity and to determine whether they have synergistic effects if combined. This type of research has been identified by the NIH as a high priority research area.

The MAX Trial has been highly successful to date. We have enrolled 123 volunteers over the first 18 months. Sixty percent of volunteers are women, and 40 percent are Hispanic or non-white. Retention has been excellent, with an 80 percent completion rate. In addition, compliance has been outstanding, with subjects attending an average of 87 percent of the exercise classes. MAX trial activities have received high ratings overall from participants (8 to 9 on a scale from 1 to 10, with 10 being the highest). We plan to close enrollment at the end of 2009 and make study results available.
“Funding from the Association has been critically important for me to begin to establish myself as a leader in efforts to identify effective interventions for the prevention of Alzheimer’s and other dementias.”

— DEBORAH BARNES, PH.D., M.P.H.

available in 2010. We also are beginning to perform one-year follow-up evaluations in MAX trial participants to determine whether the effects of the interventions are maintained over time.

Interventions such as physical and mental activity have tremendous potential to be effective weapons in the fight against Alzheimer’s and other dementias. Because the incidence of Alzheimer’s increases so dramatically with age, interventions that can delay disease onset by as little as a year or two could prevent millions of people from ever developing symptoms. Funding from the Alzheimer’s Association has been critically important for me to begin to establish myself as a leader in efforts to identify effective interventions for the prevention of Alzheimer’s and other dementias.”
The benefits of physical and mental fitness for prevention of age-related illnesses are unequivocal, and it is reasonable to assume that it would have comparable advantages for prevention of age-related neurodegeneration such as in Alzheimer’s disease. While strategies for physical and mental fitness are common in hopes of preventing Alzheimer’s, the mechanism underlying these strategies is poorly understood. Moreover, how do we ascertain the most effective tactics for preventing Alzheimer’s disease?

Animal models can be very useful for identifying key variables by which one’s environment and experience may affect the development and progression of disease. In our study, we proposed to let mice with the familial Alzheimer’s disease (FAD)-linked mutant gene APPswe/PS1ΔE9 experience a complex environment and examine its effect on the development of Alzheimer’s-like changes in the brain and on critical aspects of brain plasticity (the brain’s ability to change in response to a stimulus, such as learning). These aspects were the development of new neurons in the hippocampus, the seat of memory and learning in the brain, and long-term improvement in the communication between neurons in the brain, called long-term potentiation (LTP).

We showed that experience in a complex environment rescued mice’s ability to generate new neurons in the hippocampus. This was accompanied by significantly reduced levels of hyperphosphorylated tau protein and the oligomeric forms of beta-amyloid, precursors of Alzheimer’s disease, in the hippocampus and cortex of mice. In collaboration with Drs. Gustavo Pigino and Scott Brady at UIC, we observed that experience in a complex environment was linked to enhanced expression of neuronal anterograde motor kinesin-1, a protein that facilitates the transport of nutrients and subunits of the cell from the cell body to the synaptic terminals, the ends of axons where chemical messengers are released from one neuron,
“Our research suggests that physical and mental stimulation can rescue critical aspects of brain function that are impaired in Alzheimer’s disease and that methods that encourage brain plasticity may help prevent or treat Alzheimer’s disease. The Alzheimer’s Association was instrumental in its support and contribution to this study.”

— ORLY LAZAROV, PH.D.
In 2004, I received a Zenith Fellows Award from the Alzheimer’s Association to systematically test, for the first time, the effectiveness of counseling for couples in which the husband or wife had early-stage Alzheimer’s disease. While couples counseling is an established therapeutic modality and clinicians may offer it in their practices, it had not been rigorously evaluated in this population.

Our hypothesis was that counseling the members of the couple together would significantly improve their relationship. With the support of the Zenith Fellows Award, we developed a couples counseling intervention to focus directly on relationship distress. The intervention was tested through a pilot study at the Center of Excellence on Brain Aging at New York University’s Langone Medical Center. The goal of the intervention was to focus on the couple as a unit. Individualized and emotion-focused, the intervention was designed to support the relationship, mitigate negative effects of the illness and potentially reduce depression for both individuals. The counseling sessions provided a supportive environment in which members of the couple could share their emotional reactions to the diagnosis with each other. Helping couples address their current reactions and consider future plans and needs was one of the key roles of the counselor.

The intervention consisted of six counseling sessions in two months. Forty-one couples were randomly assigned to receive the intervention immediately (20 couples) or participate in the control group (21 couples) and receive the intervention afterward if desired. Comprehensive written assessments were recorded at baseline and at the two- and four-month follow-up sessions. After the four-month follow-up, couples in the control group were offered the intervention. All participants received ad hoc counseling from the time of enrollment. Among well spouses, 61 percent were women. The average age of the well spouses was 72.8 (range, 52–89); the average age of people with Alzheimer’s was 76.3 (range, 55–91). Assessment and intervention were available either at the Alzheimer’s Disease Center at New York University or at home; four of the 41 couples were assessed at home, where two of the couples also received counseling sessions. The overall dropout rate of 19.5 percent (eight of 41 couples) is not uncommon in this population.

The intervention had a significant effect on well spouses’ evaluation of their relationship with their partners, as indicated by the Dyadic Adjustment Scale (DAS), a well-known scale that measures relationship quality. A significant improvement in the DAS occurred from baseline to the two-month follow-up for well spouses in the intervention group compared with
those in the control group. This was maintained at the four-month follow-up. A significant difference was also seen between the two groups in the Goal Attainment Scale, with both well spouses and people with Alzheimer’s disease in the intervention group more likely to have achieved their goals at the two-month follow-up than those in the control group. Couples in the intervention group also reported improved communication.

Study participants shared their appreciation with the staff for having had the opportunity to express thoughts and feelings they previously felt were too frightening or dangerous to convey to each other. They also expressed appreciation for discovering that the relationship could continue to support the integrity and value of each member despite the impact of the illness. While goals expressed at baseline were largely achieved, new goals emerged and were addressed during counseling. Most well spouses said they were surprised at how communicative their ill spouses were during the counseling sessions. Interestingly, after participating in the intervention, 11 of the men with Alzheimer’s disease joined an early-stage support group run by one of the counselors.

Results of this pilot study suggest that providing counseling to couples while the functional impact of the illness is still relatively mild can have a significant positive effect on their relationship. Results from this Zenith Fellows Award–supported study were published in two articles appearing in the peer-reviewed journal *Clinical Gerontologist*. The high level of satisfaction that participants expressed and the positive effects of the intervention on the marital relationship suggest that additional investigation of couples counseling is warranted.”

“I received a Zenith Fellows Award from the Alzheimer’s Association to systematically test, for the first time, the effectiveness of counseling for couples in which the husband or wife had early-stage Alzheimer’s disease. While couples counseling is an established therapeutic modality and clinicians may offer it in their practices, it had not been rigorously evaluated in this population.”

— MARY MITTELMAN, D.P.H.
"Alzheimer’s disease is strongly linked to abnormal metabolism of beta-amyloid and its precursor, the amyloid precursor protein (APP). In addition to the 40- and 42-amino-acid-long forms of beta-amyloid, cerebrospinal fluid (CSF) and brain tissue contain a broad range of other beta-amyloid peptides. These beta-amyloid fragments result when enzymes called secretases clip APP. During the past year, we have confirmed earlier data suggesting that types of beta-amyloid fragments in CSF separate individuals with sporadic Alzheimer’s disease from those without Alzheimer’s with high accuracy. In addition to the well-established Alzheimer biomarker beta-amyloid 1-42, those with Alzheimer’s have high levels of the shorter fragment beta-amyloid 1-16, in CSF.

Experiments conducted in cell cultures and in mice show that this fragment is the result of alpha- and beta-secretase clipping the same APP molecule. This represents a previously unknown pathway in which APP is processed. We have recently verified this pathway in dogs. Further, we have shown that this pathway is strongly induced in response to the inhibition of the gamma-secretase enzyme in cell cultures, mice, dogs and humans. Since beta-amyloid 1-16 is water-soluble and not influenced by overall amounts of amyloid in the brain, we believe that this marker will be very useful in future studies of potential therapies that inhibit or change the behavior of gamma-secretase. We have also shown that beta-amyloid 1-16 does not have any acute toxic effect on synapses on its own.

When comparing the beta-amyloid patterns in the CSF of people with sporadic Alzheimer’s and those who are carriers of the Alzheimer’s-causing genetic mutation PSEN1 A431E, the patterns separate the two groups completely. PSEN1 A431E mutation carriers have levels of beta-amyloid 1-40 and 1-42 that are similar to people with sporadic Alzheimer’s, but mutation carriers have extremely low levels of beta-amyloid 1-37, 1-38 and 1-39. It is tempting to speculate that these fragments may inhibit the aggregation of individual beta-amyloid 1-42 peptides into multiple-peptide oligomers and that the key Alzheimer’s-promoting effect of PSEN1 A431E, and possibly several other familial Alzheimer’s disease–associated PSEN mutations, is gamma-secretase activity that results in loss of the protective beta-amyloid 1-37, 1-38 and 1-39 peptides. Modulating gamma-secretase function would in that case be a novel approach to prevent Alzheimer’s-associated beta-amyloid aggregation.
Finally, we have discovered several previously unknown N-terminal fragments of APP and APP/beta-amyloid fragments. The results described above have thus far resulted in five publications.

The Alzheimer’s Association grant was a true vitamin injection into our ongoing research examining methods to better understand the origins of Alzheimer’s. Some of these methods had been under development for quite a while. We realized their potential in shedding light on how amyloid metabolism might be changed in individuals with Alzheimer’s disease or those at high risk of developing Alzheimer’s and wrote a grant proposal describing how we would push the projects further. The reviewer comments were very positive and helpful, and the grant money was really needed. It is also notable that Alzheimer’s Association funding is quite prestigious and very well recognized in the field.”

“...The Alzheimer’s Association grant was a true vitamin injection into our ongoing research examining methods to better understand the origins of Alzheimer’s...”

— HENRIK ZETTERBERG, M.D., PH.D.
“Funding from the Alzheimer’s Association has helped advance my career in Alzheimer’s disease research on multiple fronts. First, funding from the Alzheimer’s Association has allowed us to determine that magnetic resonance imaging (MRI) can be used to detect the accumulation of gangliosides (a type of lipid associated with beta-amyloid) before the symptoms and behavioral changes of the disease develop. A paper describing the results of this work was accepted for publication in the journal *Magnetic Resonance in Medicine*, and the Alzheimer’s Association is gratefully acknowledged. Second, funding from the Association has allowed us to test new MRI contrast agents aimed at detecting the accumulation of protein and lipids in the brain, as well as molecules called ‘reactive oxygen species’ that include free radicals, and microglia, cells that normally protect neurons but that may malfunction in Alzheimer’s.

Support from the Alzheimer’s Association has also advanced my career through facilitation of collaborations and interactions with other scientists. For example, funding from the Alzheimer’s Association to Dr. Eric Klann helped foster a collaboration between my lab and Dr. Klann’s lab at New York University. As a result, we co-mentored a post-doctoral researcher whose findings will appear in the prestigious *Proceedings of the National Academy of Sciences*. Additionally, the findings from our collaboration helped this young post-doc obtain independent research funding, which she will use as a springboard to launch her own career.
I’ve also benefited from my local Alzheimer’s Association chapter in Houston and the Association’s national office, which have helped introduce me to many other scientists as well as members of the public. This combination of scientific and lay perspectives has had a tremendous impact on me professionally and personally.

More than words can say, I am extremely grateful to the Alzheimer’s Association for its grant support, the introduction to many other scientists and members of the public, and for helping facilitate productive collaborations.”

— ROBIA PAUTLER, PH.D.
Giulio Taglialatela, Ph.D.,
Associate Professor of Neuroscience and Cell Biology, University of Texas Medical Branch, recipient of a 2008 Investigator-Initiated Research Grant

“My interest in understanding the molecular and cellular events underlying the demise of nerve cells in the healthy or debilitated brain as it ages has been lifelong. Formally, it began during my graduate training at the Institute of Pharmacology of the University of Rome ‘La Sapienza’ in Italy. I still remember the words from my mentor that set the stage for my scientific career, from my postdoctoral years spent on both sides of the Atlantic Ocean in the United States and Italy, to my current position as a tenured associate professor in the Department of Neuroscience and Cell Biology at the University of Texas Medical Branch at Galveston. On one of those long-ago Roman days, I was excitedly reporting about an experiment we had conducted in which the lifespans of extremely old (and rather debilitated) laboratory animals were extended. My mentor quickly dampened my enthusiasm with a grave look and a sage warning: ‘Why add years to later life, rather than add life to later years?’

This simple notion elegantly describes the challenge faced by hundreds of scientists through several decades of research for a cure for Alzheimer’s disease. Indeed, the most devastating aspect of Alzheimer’s is the individual’s gradual loss of cognitive function, slowly chipping away at the faces, places and memories familiar to them. No one knows if halting or even reversing this cognitive decline may extend the individual’s life, but certainly it would be a formidable step toward assuring quality life in one’s later years.

With this goal in mind, my research focuses on identifying mechanisms of nerve cell malfunction in the early stages of Alzheimer’s that are responsible for the initial changes in mental function. The hope is that once detrimental processes are identified, they could be corrected with medications.

Perhaps the best characterized physical feature of brains afflicted with Alzheimer’s disease is the conspicuous presence of amyloid plaques formed by aggregates (fibrils) of individual units of the protein beta-amyloid. Although recognized for many years as a hallmark of Alzheimer’s, it was found that the number of amyloid plaques on autopsy in individuals with Alzheimer’s did not correlate with the disease severity. Instead, the levels of much smaller aggregates of beta-amyloid (oligomers) that precede the development of large fibrils and plaque formation were directly associated with disease severity. This indicated that beta-amyloid oligomers rather than later-formed fibrils may be the main offender in Alzheimer’s disease. Significantly, other studies found that beta-amyloid oligomers injected directly into the brains of healthy laboratory rodents produced severe memory deficits without inducing the death of brain cells, thus suggesting that beta-amyloid oligomers may trigger cognitive impairment through disturbing neuron function. As such, their effect on memory may be reversible if the cellular mechanisms involved are better understood.
While these seminal observations sparked a wealth of research aimed at characterizing beta-amyloid oligomers, we discovered that the levels of calcineurin, an enzyme that impedes memory formation, were elevated in the brains of mice genetically altered to produce human beta-amyloid. Elevated levels of calcineurin, the appearance of beta-amyloid oligomers, and memory deficits were present simultaneously in these mice, but months before the appearance of amyloid plaques. Most important, when we treated these cognitively impaired mice with a drug that inhibits calcineurin, their memory was restored. Could that indicate that calcineurin initiated the memory deficits associated with beta-amyloid oligomers in these genetically altered mice? And if so, could drugs that inhibit calcineurin stop or reverse this process?

Our initial results using artificial beta-amyloid oligomers published in 2008 in the journal *Aging Cell* were highly encouraging and suggested that calcineurin inhibition should be further explored as a way to alleviate cognitive impairment in the early stages of Alzheimer’s. However, proof that the presence of beta-amyloid oligomers in the brain caused calcineurin-induced memory changes was needed to push this particular field forward. One way to achieve this goal was to extract beta-amyloid oligomers directly from the brains of genetically altered mice, inject them into the brains of normal mice, and observe if 1) this resulted in elevated levels of calcineurin in the brain, 2) memory was impaired and 3) treatment with a calcineurin inhibitor would reverse these effects. This was an ambitious plan, but bore the potential for returning highly significant results if successful. In other words, this project could only be funded by an organization that would take a calculated risk to support a novel idea. And that is why we turned our attention and hope to the research grants program of the Alzheimer’s Association.

To our excitement, although not entirely surprisingly considering the long-standing track record of the Alzheimer’s Association for supporting the development of innovative research, our grant application was funded in July 2008. Experimental work begun shortly thereafter, only to be abruptly stopped on September 13th, 2008, when Hurricane Ike hit Galveston Island and our campus with unprecedented devastating force. Surging sea waters inundated the first floor of every single campus building and dramatically disrupted access to our genetically altered mice. This compromised our ability to extract beta-amyloid oligomers from these mice to test our newly funded project. However, sometimes disasters can change things for the better in unexpected ways.

In August 2008 an influential paper was published in the journal *Science* by the group led by Dr. Dennis Selkoe at Harvard Medical School. This work illustrated that beta-amyloid oligomers extracted directly from autopsied brain tissue disturbed electrical activity in neurons and disrupted memory when injected into the brains of laboratory animals. While these authors did not specifically address the mechanism involved, their results fit our hypothesis and previous rodent data like a glove on one’s hand.
Indeed, calcineurin has been known for many years to be intimately involved in regulating neuron electrical activity (the cellular basis of memory) and memory itself. Dr. Selkoe’s work further suggested to us that we could probably take a leap forward and directly test human (instead of mouse) beta-amyloid oligomers for their ability to activate calcineurin and produce memory changes that could be prevented or reversed by treatment with calcineurin inhibitors. A lack of access to our mouse colony proved to be a silver lining of Hurricane Ike’s clouds, as we shifted our focus to the mechanisms of Alzheimer’s disease in human rather than mouse models.

As a result of this new exciting evidence, and supported through the difficult months following the storm by the long-sighted generosity of the Alzheimer’s Association, we secured a steady supply of Alzheimer brain samples from two reputable national brain banks and began isolating the infamous beta-amyloid oligomers, an effort that is still under way. We also performed a wealth of biochemical analyses on these human brains that revealed signs of calcineurin over-activation similar to what we observed in the brains of genetically altered mice that express human beta-amyloid. This suggested that our hypothesis regarding what happens when beta-amyloid oligomers are present to cause toxic effects on neurons may be on target and that the elevated levels of calcineurin we found may in fact play a role in the disease.

Strengthened by these additional results, during the next two years of support by the Alzheimer’s Association we plan to complete our studies exclusively using purified human beta-amyloid oligomers. More important, we truly hope that achieving the goals of this project will thrust forward the novel concept of inhibition of calcineurin as a strategy to slow or halt the disruption of cognitive function in people with early to mid-stage Alzheimer’s disease.”

— GIULIO TAGLIALATELA, PH.D.