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## ON THE BRAIN

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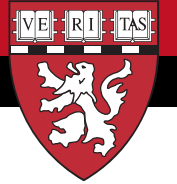
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## Gene Therapy to Bust Alzheimer's Plaques

FOR MANY YEARS, scientists have known that the brains of people with Alzheimer's disease are clogged with gooey amyloid-beta plaques that kill brain cells and disrupt memory, thinking, and other critical processes. Scientists at Harvard Medical School (HMS) recently discovered that a naturally occurring enzyme can clear the brain of these toxic plaques.

The researchers sickened laboratory mice with a gene that caused them to develop an Alzheimer's-like condition at an accelerated pace. They then used skin cells cultured from the animals' bodies to introduce the gene for "nepriylsin," a known amyloid-busting enzyme.

"Delivery of genes that led to the production of an enzyme that breaks up amyloid showed robust clearing of plaques in the brains of the mice," says Dennis Selkoe, MD, a neurologist at Brigham and Women's Hospital and the Vincent and Stella Coates Professor of Neurologic Diseases at HMS.

While this gene therapy technique provides proof-of-principle that the enzyme clears away toxic amyloid-beta molecules in the brain, Dr. Selkoe says much work remains before it could be tested effectively in humans.

### Gobbling up amyloid-beta

Nepriylsin (NEP) is a major enzyme that degrades small molecules, including amyloid-beta, whose abnormal folding in neural tissue has been implicated as a cause of Alzheimer's. Researchers in Japan and in the Selkoe lab have reported that an abundance of NEP in mice gobbles up amyloid-beta so that it forms far less toxic plaque in the brain.

Matthew Hemming, a graduate student in Dr. Selkoe's laboratory, wanted to build on that research. He created a special NEP gene, and more specifically, a protein encoded by that gene. The special NEP protein is secreted by cells and then diffuses into surrounding brain tissue.

Ordinarily, NEP is only found anchored to the surface of neurons. Hemming's and Selkoe's goal was to determine if the special secreted NEP Hemming created could diffuse outside the cell to decrease amyloid-beta build-up.

"The mechanism by which this works," says Dr. Selkoe, "is that amyloid-beta protein floats into the active site of NEP, gets cleaved [broken apart] by water, and forms smaller amyloid-beta fragments that we think are non-toxic."

### An attractive approach

In their study, the HMS scientists removed mouse skin cells called fibroblasts, made genetic changes to them, and then transplanted the modified cells back into the mice. This technique has proven effective in animal models of certain diseases, including some forms of cancer and neurological conditions such as Parkinson's, Huntington's and Lou Gehrig's diseases.

In a similar 2005 study, scientists from the University of California, San Diego found that transplanting cells secreting "nerve growth factor" (NGF, a small secreted protein that induces survival of particular neurons) into the brains of patients with Alzheimer's disease enhances the survival of an important type of brain cell that degenerates in Alzheimer's. Their study showed that NGF can help prevent the degeneration of cells resulting from the Alzheimer's disease process, including the overproduction of amyloid. These transplants may have slowed cognitive decline in some patients with Alzheimer's.

"They implanted genetically engineered skin fibroblasts, like we did in mice, and found that this appeared to have a protective effect against neuronal loss," says Dr. Selkoe.

This form of gene therapy is attractive, he adds, because it can deliver a higher dose of a protein than is possible with conventional oral medication,

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Dennis Selkoe, MD

## 'Executioner' Enzymes Link Stroke, Head Injury to Alzheimer's Disease

A CELLULAR CHAIN of events during stroke and head injury, led by so-called "executioner" enzymes, can trigger Alzheimer's disease by enhancing the formation of toxic amyloid-beta plaques in the brain, according to a recent study published by Harvard Medical School (HMS) researchers.

This finding could lead to treatments that reduce the risk of developing Alzheimer's following a stroke or head injury.

"We have discovered how a stroke [or other brain injury] can trigger a series of biochemical events that increase amyloid-beta production in the brain," says Giuseppina Tesco, MD, PhD, of Massachusetts General Hospital's Genetics and Aging Research Unit and assistant professor of neurology at HMS. "These findings raise the prospect of novel therapies that could interfere with this process and reduce the risk of Alzheimer's disease in stroke or head trauma patients."

### Shedding light on Alzheimer's vulnerability

Scientists have long known that strokes and head injuries can increase one's risk of developing Alzheimer's disease, but they were uncertain of the mechanism involved. Alzheimer's disease is a chronic brain disease in which toxic "amyloid-beta plaques" destroy brain cells responsible for memory, language, perception and motor skills. Amyloid-beta plaques form when a large protein, called amyloid precursor protein (APP), is snipped by the enzyme beta-secretase, also known as BACE. This cutting action releases fragments of amyloid-beta, the building block for the toxic plaques in the brains of Alzheimer's patients.

In earlier research, Dr. Tesco and her colleagues at Massachusetts General Hospital showed that BACE is normally broken down by lysosomes, which are like garbage disposals that grind up worn-out enzymes and other unnecessary cellular material. An enzyme called GGA3 typically gobbles up BACE and transports it to lysosomes for its destruction.

In their study, published in the journal *Neuron*, Dr. Tesco's team found that particular enzymes called "caspases" also break down GGA3. Caspases are "executioner" enzymes that mop up damaged brain cells that result from brain injuries such as strokes. Thus, following brain injury, less GGA3 is available to take BACE to its destruction in

lysosomes. This creates a vicious cycle that raises BACE levels and increases the production of amyloid-beta.

"In acute brain injury," Dr. Tesco says, "there is always caspase activation. Even with a mild concussion, caspases are activated, but in smaller amounts [than in, say, a stroke]. The activation of caspases is a critical function in cell death in head injuries and stroke."

The researchers tested this by "silencing" the GGA3 gene in cells and found that this caused increased levels of BACE and the production of amyloid-beta proteins. Further, they determined that GGA3 was broken down and BACE levels rose after inducing strokes in the rats. In postmortem analyses of brain tissue from Alzheimer's patients, the researchers also found increased proportions of BACE relative to reduced levels of GGA3.

The findings shed light on how the brain becomes vulnerable to Alzheimer's disease, especially as we grow older, since insults to the brain in the form of strokes or "transient ischemic attacks" (TIAs, or mini-strokes) can set this process off and increase BACE activity in the brain. She adds that everyone has BACE and amyloid-beta in the brain and that their presence increases with age. The goal is to determine at what level they trigger symptoms.

### Inhibiting caspases and BACE

"Knowing the molecular mechanism linking brain injury and Alzheimer's disease," says Dr. Tesco, "gives scientists a better indication for therapy targets." Given her findings that caspases activate BACE and that BACE increases levels of amyloid-beta, she adds that these are two potential targets for treatment.

Drug companies are currently in Phase II clinical trials of caspase inhibitors for viral infections and tissue damage that occurs after a liver transplant. For Alzheimer's disease, the thinking is that drugs could block the activity of caspases so they do not cut GGA3. Consequently, BACE and amyloid-beta levels would not increase. "Our findings," says Dr. Tesco, "suggest that if someone has an acute brain injury or head trauma, then blocking caspases would be ideal."

Other studies are looking at inhibiting the activity of BACE. Since BACE cleaves APP—the first step in amyloid-beta production—blocking the

action of this enzyme might prevent the production of APP in the first place. Dr. Tesco says these BACE inhibitors "provide an interesting therapeutic target."

At Massachusetts General Hospital, Dr. Tesco and her colleagues are working on animal models to determine how to prevent BACE activity and the resulting increase in amyloid-beta following head trauma.

"It takes a long time to develop these drugs [to block either BACE or caspase activity]," she says, "but

now we know the molecular mechanism linking acute brain injury and Alzheimer's disease, and we expect these drugs to be useful in subjects who have suffered a stroke or head trauma."

If scientists can find ways to protect GGA3 from caspase cleaving, they might be able to reduce the risk of Alzheimer's disease for thousands of people who suffer a stroke or brain injury every year.

## Gene Therapy to Bust Alzheimer's Plaques

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and can deliver it directly to the site in the brain where it is needed. The gene is introduced in the brain through a highly targeted surgical procedure, so it is placed where physicians think it is needed. In addition, because the patient's own skin cells are used to deliver the gene, the likelihood of a negative immune response, where the body rejects the implanted cells, is greatly diminished.

### Obstacles remain

Dr. Selkoe, however, cautions that there are drawbacks to the implantation of genetically engineered cells. Scientists do not yet know if diffusion of the active protein in the brain will cause any adverse effects. They *do* know that they will not be able to turn the gene off once it is transplanted into the brain, so if something does go wrong, there is likely no option to correct it by shutting off the gene. Further, researchers don't know if the new cells may eventually die out. If they do, physicians would have to go back in and retransplant cells expressing the gene.

"We don't know if the effect is permanent," says Dr. Selkoe. "We simply don't know how long the cells will live in the host. We also can't be sure there won't be an unwanted local reaction to the implanted cells. There are downsides to this approach, and we don't have all the answers yet."

Another obstacle is delivering the gene into an organ the size of the human brain. While the technique worked in mice brains, human brains are much larger.

"In my opinion, Alzheimer's is not the most attractive target for gene expression transplants," says Dr. Selkoe. "Local delivery [of genes] in Alzheimer's is a problem. Where in the brain do we put the gene? In the cerebral cortex [the brain's

gray matter, where information is processed]? In the hippocampus? We might get away with that, but it's risky because the hippocampus, which is important for memory, is already poorly functional in Alzheimer's patients."

For these reasons, Dr. Selkoe says the next step in this research is to investigate whether placing the NEP somewhere else in the body is sufficient to degrade amyloid-beta protein and thus reduce its levels in the brain. For example, can the NEP-expressing cells be placed in the abdominal cavity, where it is naturally synthesized by the liver, and have the same effect of decreasing amyloid-beta in the brain? If so, physicians could inject the patient's own cells safely into the abdomen or perhaps under the skin (like they do for some drugs or proteins). The cells could be implanted in an encapsulated form and could thus be removed if negative effects ensued.

"There is a free exchange of amyloid-beta protein in the circulation," says Dr. Selkoe. "There is entry and exit of the protein through the blood-brain barrier. That said, will peripheral NEP expression lower body levels of amyloid-beta enough to secondarily decrease it in the brain?"

While many questions remain about the effectiveness of this approach for clearing toxic amyloid-beta plaques in the brains of Alzheimer's patients, Dr. Selkoe says he and his team are pleased with their initial results.

"The paper doesn't mean this is the most direct or safest way to treat Alzheimer's," he says. "We wanted to prove the principle that secreted NEP works to decrease plaques in the brain. Now, we and other labs need to go forward and figure out if there are easier, safer ways to do this."



## Stopping PTSD in its tracks

**T**HE POLICE OFFICER who quits his job just short of retirement because of persistent nightmares of a bank-robbery shooting. The young rape victim who repulses every time she sees a certain brand of blue jeans. The combat veteran who awakens screaming after unrelenting images of mortar fire disturb his sleep. All three have something in common: post-traumatic stress disorder (PTSD), an often debilitating anxiety disorder that can develop after exposure to a terrifying event or ordeal in which grave physical harm occurred or was threatened.

In a recent study, Harvard Medical School (HMS) researchers have taken one step closer to new treatments for PTSD by identifying the cellular mechanism that explains why emotionally charged events are not easily forgotten. The study, published in the *Proceedings of the National Academy of Sciences*, found that a surge of the neurotransmitter norepinephrine (also called noradrenaline) plays a key role in emotional memory—and that blocking its activity might interrupt this memory process and stop PTSD in its tracks.

“We previously demonstrated that the learning of fear is associated with increased communication between neurons in the amygdala [the region of the brain where fear memories are formed] and brain regions that provide information about sound to the amygdala,” says Vadim Bolshakov, PhD, director of the Cellular Neurobiology Laboratory at McLean Hospital and an associate professor of psychiatry at HMS. “[In our study,] we found that noradrenaline facilitates this increased communication.”

Norepinephrine is a neurotransmitter released by the brain during stressful events and is involved in the “fight-or-flight” response to fear.

“Based on our findings,” says Dr. Bolshakov, “we suggest that manipulations of the adrenaline system could be used to treat certain psychiatric conditions, including PTSD and generalized anxiety disorder,” which are linked to the brain’s fear conditioning mechanism.

### How memories are formed

Memory has two stages: short-term and long-term. The initial coding of information in the brain is called “working,” or short-term, memory. This new information is held in the brain in precariously

shifting patterns of neural activity (think of nerve cells talking to one another). After several hours, the information is encoded in more persistent molecular formats as new synapses—special junctions through which nerve cells send signals to one another—are formed and memory becomes more long-lasting. This process is called memory consolidation.

According to memory consolidation theory, new memories are easily changed and subject to disruption before a series of processes in the brain makes them progressively more stable. Recall of long-term memories, on the other hand, can return memories to their previously shaky state. After recall, memories must be consolidated again or they will be forgotten.

Since recall of anxiety-producing memories is a key component of PTSD, it is necessary to understand the concept of “fear conditioning,” a mechanism by which organisms, including humans, learn to fear new, initially biologically insignificant stimuli. Fear memory is formed when a particular harmful event is associated with a specific context or neural stimulus (such as a sound or smell). Eventually, this stimulus can elicit a state of fear. During periods of intense stress, reactions to the stimulus are stored in the amygdala and can return as physical symptoms or flashbacks. While much of our natural fear conditioning is beneficial—such as learning not to touch a hot stove or ducking at the sound of gunshots—fear conditioning can sometimes become unmanageable, manifesting itself as PTSD.

### “Sound equals pain”

Norepinephrine, which is at the crux of the Bolshakov study, is released in the brain and plays many complex roles. One of them is to “help us acquire memories with emotional significance,” Dr. Bolshakov told *Newsweek* magazine in a recent online interview. “It’s why you are likely to remember what you were doing on September 11, 2001.”

During his study, Dr. Bolshakov and his colleagues conditioned rats to respond to an auditory stimulus related to a traumatic event (in this case, a foot shock after hearing a loud noise). The animals, he says, remembered that “sound equals pain.”



The researchers infused slices of amygdala from the rats’ brains with a solution containing norepinephrine and measured electrical currents flowing between nerve cells during the foot shock. Most cells in the amygdala are neurons that receive incoming information about the characteristics of auditory signals during fear conditioning. The researchers discovered that norepinephrine increases the transmission of acoustic information to the amygdala, allowing signals to flow more freely and enhancing fear memory.

### Blocking norepinephrine activity

Since scientists already know that reconsolidation can bring memories back to their original unstable state, Dr. Bolshakov and his colleagues believe that blocking the actions of norepinephrine may prevent emotional memories from becoming long-lasting in the first place. Such interventions would interfere with receptors for the neurotransmitter in the amygdala and reduce the fear memory enhancing effect of the neurotransmitter.

“We can take long-term memories and ask people to recall them so they become as new again, and thus shaky and unstable for several hours after memory reactivation,” says Dr. Bolshakov. “This is the science behind the idea of

norepinephrine blockers. After recall, the patient is given a blocker to prevent the memory from becoming long-term.”

These blockers could be given as soon as possible after the trauma or after a retriggering of PTSD, when memories are moving from their short-term state to long-term again.

“This is what makes this approach so attractive,” says Dr. Bolshakov. “Ideally, we could treat PTSD a long time after the event occurs.”

A hypertension drug called propranolol is being used experimentally—and with some promising results—in emergency rooms to block norepinephrine activity in trauma patients. Dr. Bolshakov says another hypertension drug, Prazosin, is also a possibility. “We know these drugs do something besides reducing hypertension,” he adds. “They affect neurons in the brain, blocking receptors for norepinephrine.”

The work on norepinephrine blockers in humans is still in its preliminary stages. If Dr. Bolshakov’s hunch is right, however, and drugs can be developed to block the activity of norepinephrine, people who suffer from PTSD may be only a simple treatment away from ridding themselves of these terrible memories.



Vadim Bolshakov, PhD

## Genetic Differences Identified in Schizophrenia and Bipolar Disorder

SCIENTISTS HAVE KNOWN for years that there are similarities between schizophrenia and bipolar disorder. They share many of the same symptoms, similar treatments, and a common molecular trigger.

For the past 25 years, cell biologist Francine Benes, MD, PhD, has studied the cellular and molecular basis of these two brain diseases. She and her colleagues at McLean Hospital have now identified clusters of genes in the brains of patients with schizophrenia and bipolar disorder that may help explain the differences between the two psychiatric disorders.

"We cannot say for sure that these are the genes that cause the illnesses," says Dr. Benes, director of McLean's Program in Structural and Molecular Neuroscience and the William P. and Henry B. Test Professor of Psychiatry at Harvard Medical School, "but it seems likely that...they may be related to [a person's] susceptibility to... the disorders, and that is important."

The findings, published in the *Proceedings of the National Academy of Sciences*, may lead to new, more effective treatments for each disorder. Today, many of the same drugs are used to treat certain aspects of both illnesses, often ineffectively.

### Controlling the flow of information in the brain

Schizophrenia is a chronic, severe, disabling brain disorder characterized by hallucinations, paranoia and bizarre delusions. Bipolar disorder, also called manic-depressive disease, is a brain disorder that distorts moods and thoughts, destroys the basis of rational thought, and often erodes the desire and will to live.

Previous studies have shown that defects in the function of "GABA" cells in the hippocampus, the part of the brain responsible for memory and learning, are present in the brains of patients with schizophrenia and bipolar disorder. GABA is a neurotransmitter that helps control the flow of information along complex brain circuits.

Throughout the brain, cells release GABA, helping to control the flow of information along complex cognitive, motor, emotional, and other circuits. To understand how GABA works, think of being in a crowded room with a lot of talking. Normally, your brain can damp down surrounding

noise to focus on the conversation in which you are participating. The brain needs increased GABA cell activity to filter out all this information. People with schizophrenia, however, can't filter out the excess noise, so they get overwhelmed with too much information to process. People who do not have the disorder use GABA to filter out background noise, while those with schizophrenia don't have sufficient GABA-filtering activity.

Earlier studies have shown that there is decreased expression (gene function) of GAD67, an enzyme that is produced by GABA, in the brains of patients with either schizophrenia or bipolar disorder. Dr. Benes' study sought to determine what caused this decreased expression.

Using postmortem brains of patients with schizophrenia and bipolar disorder, the researchers extracted parts of the hippocampus where GAD67 is known to have the least expression. They then extracted RNA and conducted an analysis that looks at particular genes and tells scientists what other genes interact with them. In this case, the analysis identified which genes interact with the regulation of GAD67.

Dr. Benes and her team identified a cluster of 25 genes that are involved in the regulation of GAD67 and found that GAD67 shows significantly decreased expression in both disorders. Ten of the genes showed significant changes in expression in the brains of patients with bipolar disorder,

while 12 others showed changes in the brains of those with schizophrenia. The genes all came from the same population of GABA cells; however, the cluster identified in the schizophrenic brains was different from the cluster in the brains with bipolar disorder.

"The same set of genes came forth for both disorders," says Dr. Benes, "The cells had common physical characteristics, but the way the cells functioned was fundamentally different"

### Dysfunctional GABA

In the case of schizophrenia, Dr. Benes and her team identified genes with defective "on/off" switches. Two of these schizophrenia genes were overexpressed, giving the researchers a potential molecular mechanism to examine.

Another surprise finding, Dr. Benes says, is that the genes involved in bipolar disorder are ones that are active in the early development of the brain. Their findings suggest that, in bipolar disorder, GABA neurons may revert to a less mature state than is usually encountered in adulthood. Knowing this may help scientists develop molecular strategies that increase the expression of GAD67 and thus increase GABA cell activity in the brain.

Dr. Benes says the findings suggest that the different patterns of expression may reflect the differences in genes that make a person susceptible to either disorder in the first place.

### Specific treatments for specific diseases

By identifying potential genetic targets for these brain disorders, scientists may be able to develop more effective treatments based on the particular cellular and molecular mechanisms at play in each disease.

"From the standpoint of clinical care," says Dr. Benes, "we need more specific forms of therapy. In order to do this, we need to understand these illnesses at the molecular and cellular level. The findings reported in this paper bring us closer to realizing that goal."

Dr. Benes and her colleagues have another paper due to be published in early 2008 that provides more details about GABA cell activity within specific neural circuits. The research demonstrates that GABA cells in key brain circuits in schizophrenia behave fundamentally differently than they do in bipolar disorder. The difference, she says, impacts the flow of information in the hippocampus and may give researchers other avenues to approach in finding effective treatments for these disorders.

While Dr. Benes says, "we can't think in terms of being close to identifying the genes involved in schizophrenia and bipolar disorder," her study is leading toward a better understanding of the differences in gene expression that help define each illness and distinguish them from each other.

"We don't yet understand the complex cellular and molecular pathways that are involved," she says. "The brain is too complex, with thousands of neurons and specific neuronal subtypes. We need to go back to the level of the circuitry and understand how GABA cells are influencing the flow of information through the hippocampus to other regions of the brain."

Dr. Benes' findings have pushed science one step closer to the goal of understanding of these brain disorders and one step closer to better treatments for bipolar disorders and schizophrenia.



Francine Benes, MD, PhD

