



Gray Matters

Old brain cells don't die. They just malfunction, concludes Hopkins psychologist Michela Gallagher. This picture of the aging brain could spark new strategies for preventing memory loss.

By Michael Purdy
Illustration by Marc Mongeau

An isolated, island-like community reaches a certain point in its development, and suddenly the population starts to shrink. There will be no replacement members for the community--it's downhill from here on out.

Sound like the latest iteration of the television show "Survivor"?

It's also pretty close to what neurologists formerly believed would happen to the cells in an aging but healthy human brain.

Researchers had good reasons to suspect a significant portion of the brain's nerve cells were dying off. Even if pathological conditions like Alzheimer's disease and stroke were exempted, memory impairment and slowness in thinking were clearly becoming more common as the average lifespan increased over the course of the past century. Also, scientific measurements had shown that the aging brain seemed to shrink in volume.

In the past five years, though, a new picture of the aging but healthy brain has won out over the old. The most important question for researchers now isn't how many contestants leave the island--it's how the passage of time can make some of the contestants cranky and dysfunctional.

There may be quite a few ways that healthy aging can disrupt the function of the many different types of brain cells. But according to one of the leading researchers who helped formulate and prove this new picture of the aging brain, the fact that brain cells linked to memory are malfunctioning instead of dying off is nothing less

than wonderful news.

"This is terrific, because if neurodegeneration is not a primary cause of the impairment that we see in the elderly, and if we don't have to find ways to replace missing nerve cells [through] nerve cell transplants and so forth, then the challenge of maximizing the potential of the elderly suddenly becomes much more tractable," says Michela Gallagher, professor and chair of the **psychology** department in Hopkins's **Krieger School of Arts and Sciences**.

Gallagher uses rats that are specially bred to age well and follows them over the course of their lifetimes, keeping them free from exposure to germs and common viruses.

Photo by Jay Van Rensselaer



Gallagher and her colleagues have already identified two problems unique to the circuitry of the age-impaired brain that might lead to future possibilities for pharmacological therapy. Her dedication to applying the latest research techniques from biology, psychology, and cognitive science, plus her leadership on a multimillion-dollar, multi-institutional grant from the National Institute on Aging, has made her a renowned figure in efforts to understand the aging brain.

"At this point in time she's among the top five people in the world in terms of the potential for making some major insights into the neurobiology of aging," says Kay Lund, a professor at the University of North Carolina at Chapel Hill and a co-investigator with Gallagher on the NIA grant.

One factor that makes aging and the brain a unique area of research is the longevity of nerve cells.

Even after the growth of the body stops, most cells that start to wear out can simply divide and produce duplicate replacement cells. Brain cells and other nerve cells, though, are "post-mitotic." Beyond a certain point in development, almost all of them cannot reproduce. While there are old nerve cells throughout the body, the brain is by far the most concentrated collection of these old cells.

To study the senescence of this mob of cellular Methuselahs, Gallagher developed a model of natural age-related memory impairment in rats. Working with animals instead of humans has allowed her to overcome several challenges.

"First of all, there's the whole problem that people don't let you test their memory and then take their brains," jokes Gallagher, who is a wiry, ebullient bundle of energy.

Neuroimaging and post-mortem study of the human brain can help

quantify aging's effects. However, these techniques can also fall victim to other problems. Most prominent is the need to sort out the change brought on by normal aging from that created by pathological conditions.

The earliest scientific assessments that suggested brain cell loss was a common phenomenon in aging were performed in the 1950s, when methods for detecting conditions like Alzheimer's disease were even more primitive than they are today. Some researchers expressed concern decades later that early brain volume studies might have included results from Alzheimer's patients, who do lose large numbers of brain cells to the disease.

The debate intensified with research published in 1984 showing that individual brain nerve cells shrank in volume as they aged. Was this shrinkage of the brain a product of the loss of brain cells, or of the shrinkage of individual cells that still remained?

Gallagher and others began to bring the debate to a close in the mid-1990s with startling new evidence from their animal models of aging.

At age 25 months, rat #110-33 is clearly entering his "golden months." Median life expectancy for his breed of rat is 25 to 30 months. On average, half of rats like him will have died of natural causes by the time they get to be 25 months or a little older.

He's about the size of an adolescent rabbit. From a rat's point of view, he looks good. Like other rats his age, he's superficially indistinguishable from a rat half his age.

He's been living a charmed life. Not only has he been around quite a bit longer than the average laboratory rat, he's also received care above and beyond the normal high standards applied by animal researchers. And he has been maintained free from exposure to germs and common viruses.

The only hitch in the whole experience has been a ritual his keeper imposes on him. Every so often the keeper comes and takes him from his room to another room. He's lifted from his cage, given a soothing back rub for a few moments, and then lowered into a deep pool of water.

The water's warm, about 28 degrees Celsius (82 degrees Fahrenheit). Like any healthy rat, rat #110-33 is a fine swimmer. All in all, though, he'd rather not be swimming.

Since the water is bordered by a circular, vertical wall, the only option for getting out is a platform that he remembers from prior experience. It's usually hidden just beneath the surface of the water, where he can't see it. He can, however, see shapes high above him, and over the course of several seconds he uses these shapes to find his way to the platform, gets out, shakes himself,

and waits for his keeper to take him back to his cage.

Rat #110-33 has to go through a few more trials, and his scores still have to be analyzed and averaged, but it seems pretty clear to his keeper, research assistant Leigh Ruane, that he's one of the lucky rats that make it into old age without memory impairment. His not-so-fortunate counterparts have a different experience in the tub, swimming aimlessly around the edge of the pool in a vain attempt to find their way out.

Ruane runs the Morris Water Maze for Gallagher. Named for researcher Richard Morris, who developed the test in the early 1980s, the maze consists of a circular metal basin of water about two meters wide, surrounded by a high curtain. The walls of the basin and the interior are painted white, and a white pigment has been added to the water, making it milky and opaque. At the front of the basin is a cord wrapped around a metal knob on the curtain frame. Ruane uses this cord to raise and lower the platform for different tests.

She has cut out four different abstract shapes from heavy black paper and pinned them at equal distances from each other on the curtain surrounding the maze. The shapes are about head-high to a human, seemingly too far off to do the rat any good. But Gallagher explains that the angle of the rats' heads when swimming makes this the area where they'll be looking.

Gallagher has invested many years in making her rat model one of the most meticulously prepared and thoroughly controlled efforts to identify the tangible roots of normal memory loss.

"To start, we chose Long-Evans hooded rats, which are specially bred to age well," says Gallagher. "They have a very low incidence of age-related conditions."



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As they age, the rats are exposed to several versions of the Morris Water Maze. In the most common version, a rat is inserted in the maze at a randomly determined point and trained through exposure to use the visual cues pinned to the curtain to remember the location of the hidden platform.

A computerized system above the maze automatically tracks the rats, records their progress on videotape, and transfers the data to a computer program that analyzes the effectiveness of each rat's efforts. If the rat fails to find the platform within 90 seconds, he is shown to the platform.

To make sure the rats feel motivated to get out of the water and are not impaired by physical disability or vision problems, Ruane will regularly perform one trial in which the platform is raised and clearly visible above the water. In a third type of trial, the platform is lowered deep into the water where the rats can't reach it. Watching how long the rats linger in the area where the platform is

supposed to be further supports the idea that the rodents are using what they remember to try to solve the maze.

It's an expensive way to study the problem, Gallagher notes. "You have to create and incur the costs of the entire lifespan of an animal population," she says. "But even so, given the potential insight they can provide, it's surprising how relatively little research there is on this subject in animal models."

Gallagher's investments in the animal model have paid off well. One of the rat model's first landmarks was the finding that 40 percent of aged rats clearly show age-induced brain impairment, while a lucky few score as well as the best young rats.

Aging's effects in humans are similar. About 40 to 50 percent of the elderly who do not develop pathological conditions will still suffer from age-based memory impairments, but the fortunate remainder will dash through their retirement years with their brains as nimble as they've ever been.

With that initial connection to normal human aging processes established, Gallagher and former student and longtime collaborator Peter Rapp, associate professor in the Kastor Neurobiology of Aging Laboratories at the Mount Sinai School of Medicine, stepped into the debate on memory impairment and nerve cell loss.



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Gallagher and Rapp focused on the hippocampus, an area of the brain that belongs to the limbic system. Several historical experiments had found evidence suggesting that the hippocampus was essential to learning and memory, including a case of a human patient with an injured hippocampus who couldn't form new memories.

Applying a new and more accurate technique called stereology to the problem of counting brain cells, Gallagher and Rapp compared the number of hippocampal brain cells in aged rats with memory impairment to those in unimpaired aged rats and young rats. They found no significant difference between any of the three groups.

When they published their results in the *Proceedings of the National Academy of Sciences* in September 1996, they received a swift response in the letters section of *Science* from scientists who had argued for brain cell loss. These critics challenged the importance of the PNAS results. In response, Gallagher organized a consensus letter that ran in *Science* a month later and was signed by several of her colleagues, and even by scientists who had earlier argued for brain cell loss.

Rapp feels the debate was misplaced. "Whether older literature was wrong or not was a little bit tangential to the importance of [our stereology] studies," he says. "The importance of that work was to say that one can find substantial age-related cognitive decline in

the absence of neuron loss."

Adds Lund, Gallagher's friend and collaborator, "What's important about the paper is that it really changed how we thought about age-induced decline. We're not looking for cell death, but functional decline. That means we're on a whole different research enterprise now, and it may be a lot easier to help something functionally improve than to stop it dying."

As nerve cell loss fell from favor as a cause of age-induced memory impairment, scientists began to search for its successors. To identify potential causes, Gallagher and her colleagues have been conducting detailed comparisons of the differences between the brains of aged rats with memory problems, aged rats with no memory problems, and young rats. In an average study, they compare eight to 10 young rats with 15 to 18 aged ones.

In a 1999 paper, Gallagher and her colleagues found signs that some nerve cells in the hippocampus might, metaphorically speaking, be getting a bit hard of hearing.

Nerve cells communicate with each other across the synapse, a small empty space where the cells almost but do not quite touch. The sending nerve cell releases neurotransmitters, compounds that drift across the gap in the synapse and bind to receptors on the surface of the receiving nerve cell. The "message" arrives when the neurotransmitter binds to the receiving neuron and triggers a series of reactions that cause changes inside the neuron.

Gallagher's lab found evidence of malfunction in a key component of the machinery that transfers the signal inside the nerve cell. The message still reaches its audience, but the listener is starting to go "deaf."

"The receptors involved in this signaling pathway are also connected to neural plasticity," Gallagher says. "That's a way nerve cells can change the ease or difficulty of passing a message through the synapse, and it's thought to be an important method for encoding information."

Last year, Gallagher's lab found evidence that nerve cells in an area of the brain known as the entorhinal cortex might be having problems "speaking" into the synapse. "This is an area of the brain that is a primary conduit of highly processed information from all cortical areas into the hippocampus," Gallagher explains, drawing a diagram that shows information coming into the curved hippocampus.

Information comes in from the entorhinal cortex, progresses through several different layers of two types of brain cells in the hippocampus, and comes out again into an entry point in the hippocampus to head back out into the cortex.

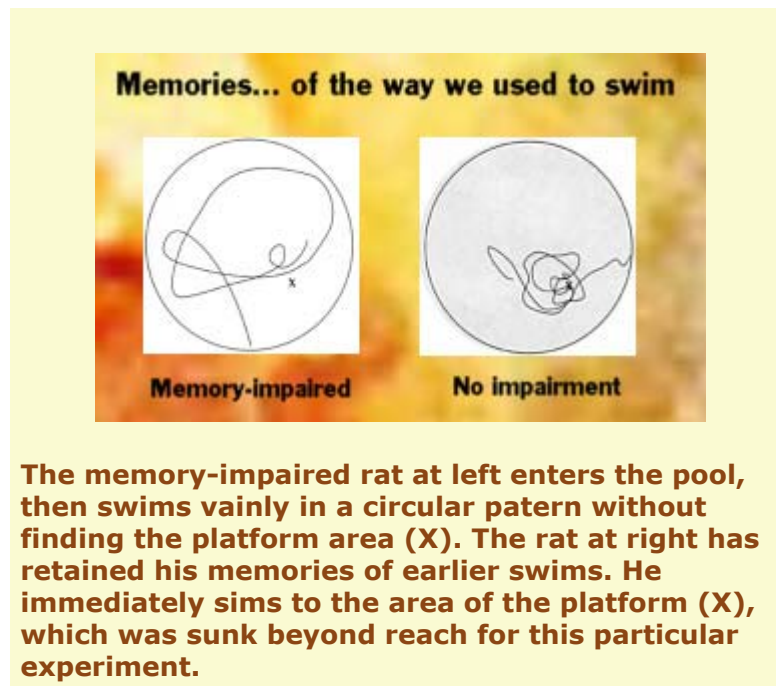
Gallagher showed that the entorhinal cortex in age-impaired rats had low levels of a protein called synaptophysin. This protein is a prominent ingredient in vesicles, the envelopes that carry neurotransmitters to the synapse. Having less of it around probably reduces a neuron's ability to communicate across the synapse.

"The integrity of the connection between the entorhinal cortex and the hippocampus starts to suffer, and as a result so does the hippocampus's connection to the cortex," says Gallagher. "And that's a problem that we don't see in age-unimpaired rat brains or in young rat brains."

The cells where synaptophysin reductions showed up, a group of entorhinal cortex cells known as layer 2 neurons, are interesting for another reason. "When patients have died of other causes and an autopsy reveals undiagnosed Alzheimer's in its earliest stages, the tangles that are a prominent early feature of the disease are localized to layer 2 neurons in the entorhinal cortex," says Gallagher. "So something going on in normal aging may be helping to set the stage for Alzheimer's disease."

On a warm, windy spring morning, Gallagher has just finished putting in for the renewal of the \$6.5 million National Institute on Aging program grant that she has shepherded for the past 10 years. The grant involves principal investigators from several different specialties and several different research institutions across the United States, and Gallagher is pointing out how similar this collaboration is to the vision of a "university without walls" advocated by Hopkins President William R. Brody a week earlier in his 125th anniversary address.

Gallagher has just completed a six-year term as the editor of the journal *Behavioral Neuroscience* and is wrapping up her first year as chair of the Psychology Department. She signed three new faculty to the Psychology Department in the past several weeks. She's getting ready to apply to her rat model the cutting-edge technique of gene array, which will test cells for the activity of millions of known and unknown genes. And she has to head out of town again to give another talk on her research.



"I don't know when she sleeps," says postdoctoral fellow Jennifer Bizon. "She's constantly trying to work in the newest technologies to ask questions that we previously hadn't been able to get to."

Lund finds that "it's really hard to stress Michela or get her disorganized. She has an ability to think about and do different things at the same time."

Gallagher admits mild concern that her energy and her devotion to being an "integrative neuroscientist" (the neuroscientist's version of a "jack-of-all-trades") might cause some of her colleagues to regard her as "hyperactive." "It's something that's needed, though," she concludes. "The investigators in my NIA program grant count on me to be able to understand across the whole spectrum of the work. To have a university without walls, you need an interpreter, a facilitator who can work across the disciplines."

University of Southern California neuroscientist Richard Thompson might have put it best when he described Gallagher as a "catalyst" for the neurosciences--somebody whose very presence makes things happen.

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▶ [Return to June 2001 Table of Contents](#)