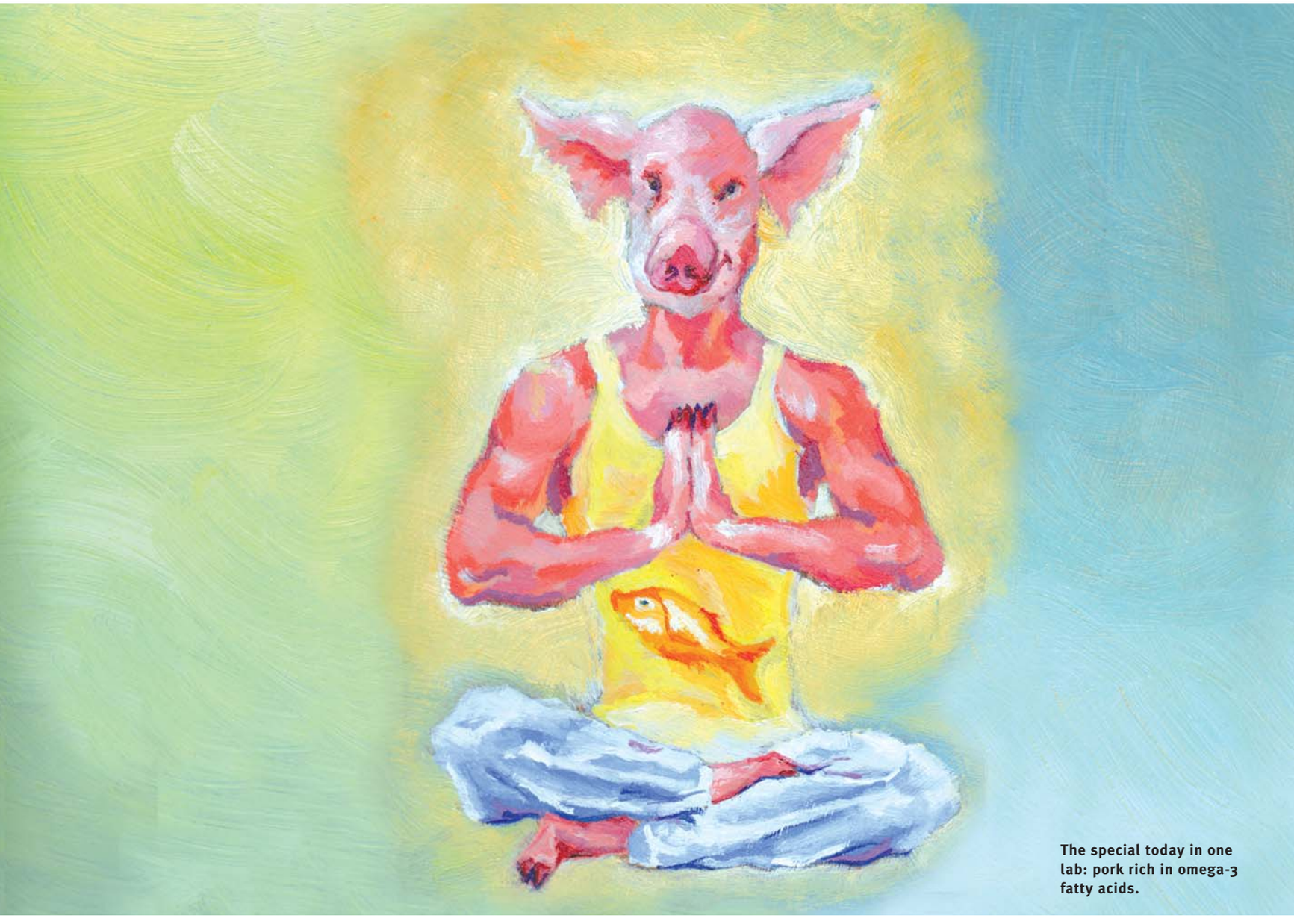


The special today in one lab: pork rich in omega-3 fatty acids.

SOME PIG!

THE ALPHA AND OMEGA OF OMEGA-3 FATTY ACIDS

BY JOE MIKSCH

Dragging a makeshift anchor in its wake, the brakeless monorail hurtles through Springfield at speeds of up to 180 mph. The conductor, one Homer J. Simpson—cartoon icon, Duff Beer connoisseur—awaits death.

Suddenly, the anchor hits a snag—the Lard Lad donut shop sign. Latching onto an enormous representation of a donut, the monorail comes to a halt. Homer, his passengers, and the rest of the town are safe.

Reflecting, Homer utters these immortal words: "Ah, donuts. Is there anything they can't do?"

Replace "donuts" with "omega-3 fatty acids," and you may be onto something. Work at the University of Pittsburgh shows that in addition to omega-3s' recognized benefit to cardiovascular health, these vital fatty acids appear to improve mood and kill liver cancer cells. Another Pitt project could help get the critical fatty acids on the traditional American breakfast table.

Tong Wu's lab has found that omega-3s appear to have an inhibitory effect on the growth of liver cancer cells. Wu, an MD/PhD associate professor in the Division of Transplantation Pathology, says that for some time, omega-3s have been known to inhibit certain kinds of cancer cells.

Wu and Kyu Lim, a PhD research scholar in the Department of Pathology, decided to look at liver cancer cells, hoping to identify the mechanism by which omega-3s fight cancer. They found that these essential fatty acids, which are not produced by the body, induced programmed cell death in liver cancer cells. Additionally, they discovered that omega-3 treatment reduced the presence of beta-catenin, a protein found in overabundance in tumors.

Their findings, Wu says, show that omega-3s operate on at least two pathways as they control the growth of liver cancer cells.

As a control, the investigators treated another set of liver cancer cells with omega-6 fatty acids, which are found in vegetable oil and are known to contribute to cardiovascular ailments. The cells, like those treated with omega-3s, stewed in their fatty acid bath for 12 to 48 hours. The researchers observed no significant changes to the cells.

Wu and Lim plan to extend their research into animal studies.

"This could be a good treatment for patients who cannot tolerate other chemotherapeutic agents," Wu says, "and may be used in combination with other therapies."

Sarah Conklin notes that omega-3s not only help the body, they can help the mind. She is a postdoctoral scholar with the Cardiovascular Behavioral Medicine Program in the Department of Psychiatry.

Omega-3s are thought to alleviate symptoms of those with severe mental disorders. Yet little research has been done looking at what role they might play in sustaining the mental health of adults.

As a graduate student at Baylor University in Waco, Texas, Conklin worked on studies showing that anticonvulsant drugs reduced aggressive outbursts in men. But the side effects of such drugs, she says, were troubling. After she read a study equating a combination of multivitamins and omega-3s with reduced aggression in prisoners, Conklin became interested in learning more about how diet affects mood and behavior.

When she came to Pitt, Conklin and her coinvestigators (including mentor Matthew Muldoon, associate professor of medicine) studied 106 healthy volunteers. Those with lower blood levels of omega-3s were more impulsive and had a negative outlook.

Conklin believes that increased levels of omega-3s balance out omega-6 fatty acids, which are known to have inflammatory effects on cells. Inflammation generates an immune response, she says, and overactive immune responses have been implicated in various disorders, including major depression.

Although none of these investigators is ready to promise that omega-3s are a cure for anything, all say that they are potentially palliative for much more than cardiovascular ailments. One day, consumers may be able to gain such benefits from—of all things (and this would appeal to Homer Simpson's palate)—bacon.

How so? Well, Pitt researchers have helped genetically engineer pigs to produce omega-3s typically derived from fatty fish and some nuts.

Yifan Dai, associate professor of surgery and member of the Thomas E. Starzl Transplantation Institute, was intrigued by the work of Massachusetts General Hospital researcher Jing Kang. Kang had transferred a gene called fat-1 into mice, which resulted in their converting unhealthy omega-6 fatty acids into beneficial omega-3s.

The MD/PhD thought that if he could make this work in larger animals—namely livestock—perhaps we could get more omega-3 into our diet without having to rely on fish. In 2004, Dai began transferring the fat-1 gene into pig cells. But to get real, live swine he called upon Randal Prather, leader of the pig cloning center at the University of Missouri–Columbia.

Prather removed DNA from pig eggs and inserted Dai's cells.

He ended up with five pig litters. One piglet was found to be a fine producer of omega-3s. Five of its clones became a breeding line of omega-3 rich pigs. The results of this work were published in *Nature Biotechnology* this spring.

"For agriculture, I think this will be very useful," Dai says. "Not only for nutrition, but if these pigs are healthier than others [their omega-3–rich bodies could ward off heart disease], it may reduce costs for farmers." He adds that for scientists, the animals could be excellent research models.

Omega-3–rich pork rinds are a way off, Dai says. First, the genetically modified pigs must win FDA approval. And other questions may arise: Will palliative pork appeal to taste buds? Will people be hungry enough for omega-3s to eat engineered pigs? ■

HELEN GRIFFIN



ALFRED PASIEKA / SCIENCE PHOTO LIBRARY

PIECING TOGETHER PARKINSON'S

A STUDENT RESEARCHER LANDS IN THE RIGHT PLACE AT THE RIGHT TIME

BY ELAINE VITONE

A man moves slowly, stiffly, and yet when his limbs are at rest, they quiver. When he wants to move, he can't. When he doesn't want to, he can't stop. Cells in his brain are dying at an alarming rate, and no one knows why. A University of Pittsburgh med student has positioned himself well to help figure out why this cellular slaughter happens in patients with Parkinson's disease.

There are two basic forms of Parkinson's—young-onset, a rare form affecting people younger than 40 that tends to run in the family, and late-onset, a common sporadic condition the incidence of which is higher in later life. The result is the same for either: progressive, wholesale destruction of cells in many parts of the brain, including those that produce dopamine, a chemical critical to fluidity of motion.

Thus far, at least two things are certain. For one, the disease has a genetic component. Young-onset cases have been linked to the inactivity of genes known as DJ-1, pink1, and parkin and to abnormal metabolism of the neural protein alpha-synuclein. For another, certain toxins such as pesticides and industrial chemicals play a role in late-onset Parkinson's. Scientists believe that most Parkinson's cases result from a combination of environmental and genetic factors; however, little is known about exactly how they work together to cause cells to die.

This summer, third-year Pitt med student Vipul Shah began a two-month break from his rotations to conduct research under the guidance of Edward Burton, assistant professor of neurology and of molecular genetics and biochemistry. Shah hopes to help broaden our understanding of the causes of Parkinson's—and satisfy his scholarly-research-project requirement at the same time. For the project, he'll continue to meet with Burton and other mentors throughout his Pitt med schooling and eventually present his results to an executive committee of faculty members and deans.

Shah reflects on the disease: "There's a lot of work that needs to be done. … It's unclear whether every single [form of Parkinson's] has the exact same pathogenesis. We're trying to figure out how they interrelate."

Burton says that the Pittsburgh Institute for Neurodegenerative Diseases (PIND) is an ideal setting for Shah's explorations. "We're in the right place at the right time."

Historically, coming up with an animal model for Parkinson's has been tricky: How do you create Parkinson's in a rat if you don't know what causes it in humans? Previous attempts to simulate the disease involved poisoning the animals with potent neurotoxins that target the dopamine-producing part of the brain. Because this method killed off all the neurons at once rather than gradually, the progression of the animals' symptoms was a far cry from that of humans with Parkinson's.

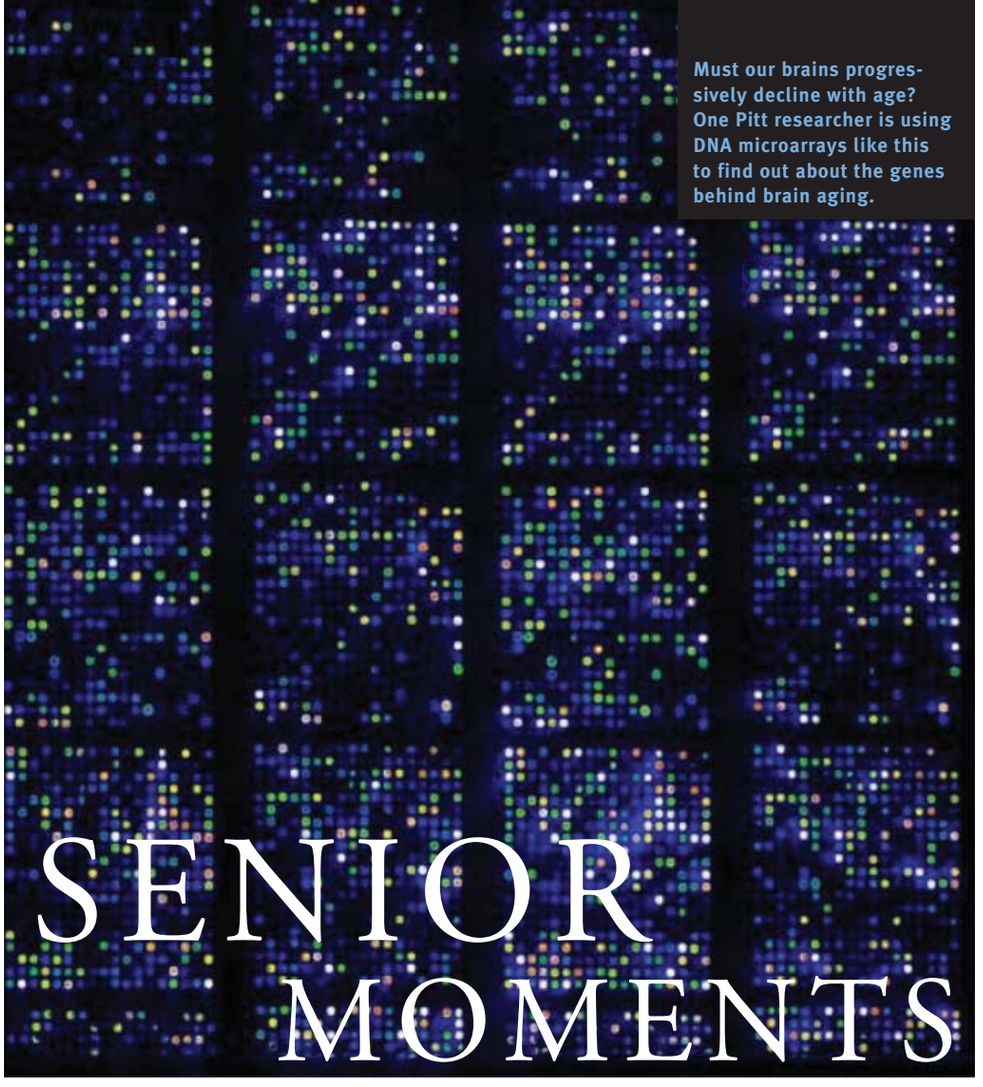
In the past two decades, several labs have shown that Parkinson's patients carry defects in their mitochondria, a key discovery that led PIND director Timothy Greenamyre to, in a sense, build a better rodent. As a faculty member at Emory University in 2000, Greenamyre found that when animals are chronically exposed to low levels of the pesticide rotenone (which is toxic to mitochondria), they develop abnormal brain pathology and progressively worsening symptoms that mirror those of human Parkinson's patients. His rotenone model—which six years ago was the first confirmation of the long and widely held suspicion that pesticides can induce the disease—is ideal for testing questions Burton's lab is exploring.

Shah will use genetically engineered viruses on Greenamyre's rats to explore these questions: How do genetic and environmental factors relate in Parkinson's? Can you prevent or treat environmentally induced Parkinson's by overexpressing parkin, DJ-1, or pink1 or by removing alpha-synuclein?

Shah likes the way Burton is open to input. The two meet regularly, but how Shah goes about obtaining usable data is largely up to him. For example, at first they debated whether Shah's project should concentrate on in-vitro or in-vivo experiments. "I am really interested in the behavioral experiments that may allow us to see actual Parkinsonian behavior in rats," says Shah, "so that is the path we are pursuing."

Perhaps giving Shah that freedom is easy enough. Shah worked in an Alzheimer's lab as an undergraduate at the University of Florida, which was where he first developed an interest in neuroscience.

"He's incredibly independent as students go," Burton says. ■



Must our brains progressively decline with age? One Pitt researcher is using DNA microarrays like this to find out about the genes behind brain aging.

COURTESY U.S. DEPARTMENT OF ENERGY

SENIOR MOMENTS

CAN SCIENCE END THEM?

BY JIM SWYERS

As we age, many of us begin to notice that our memories aren't what they used to be. We forget the names of people we've just met, regularly misplace our glasses or car keys, or lose the ability to store and retrieve numbers the way we used to.

We often dismiss these instances of forgetfulness as "senior moments" and move on with our lives. We accept that a progressive decline of memory, as well as other cognitive and motor abilities, is inevitable, like death and taxes. It seems there's nothing we can do about it.

However, according to research being conducted by Etienne Sibille, a PhD assistant professor of psychiatry at the University of Pittsburgh, such notions may not be accurate. His work is demonstrating that many long-held conceptions about aging, particularly the notion that irreversible and generalized biological decline accompanies normal aging, are dead wrong.

Using DNA microarrays—a technology that allows researchers to read the activity, or expression patterns, of all 22,000 genes in each cell simultaneously—Sibille was surprised to learn that only a small number of genes are involved in the biological decline that can be associated with an aging brain.

Sibille's team made this discovery by analyzing preserved brain tissue samples from autopsies of 39 people who ranged in age from 13 to 79. The analysis found changes in gene expression patterns in only a small subset—about 10 percent—of the total genes in the brain cells.

According to Sibille, these findings suggest that the aging process in the brain is limited in its scope: "We thought that the genetic signs of aging in the brain would be more complicated and involve many more genes. Because only a limited number of genes are involved, it means that aging in the brain is a selective event."

More importantly, Sibille believes the process may be malleable. Indeed, he and his colleagues found different patterns of age-related genetic changes in glial and neuronal cells—the two main types of brain cells. Genes in glial cells became more active with age, and genes in neuronal cells became less active.

"The fact that it is limited to a relatively few number of genes and possibly cells, and that the effect is different in the types of brain cells involved, may allow us to delay and, possibly, alter the aging process in the brain," Sibille says.

It's a bold thought. Yet Sibille believes he has evidence that such a scenario is possible. When his laboratory researchers began examining age-related genes they'd identified, they found that many of the genes were involved in the regulation of mood. By disabling, or "knocking out," the same genes in mice, they discovered one that, when absent, initiated the aging process. It turns out that the gene, called Htr1B, codes for a receptor that interacts with the neurotransmitter serotonin.

"It has long been known that there are changes in the serotonin system with aging, which are considered [to contribute] to the development of age-related mood disorders, such as depression," notes Sibille.

"When we knocked out the gene for this receptor in mice, it caused an early onset of age-related behavioral deficits."

Sibille says that by manipulating this receptor, his lab showed it was possible to "alter the trajectory of age-related events."

Much more work remains to be done before the full story of aging in the brain is known. Meanwhile, Sibille's lab is continuing to disable genes in mice that appear to be involved in brain aging and observing the subsequent behavioral changes. The investigators also are using microarrays to see what happens to the rest of the genes when some of these age-related genes are disabled.

"We need to keep deciphering what these genes are telling us to get the complete picture of what is happening at the cellular level," he says.

He believes that in the not-too-distant future it may be possible through lifestyle or drug interventions to delay the onset of the brain-aging process in humans. Even a delay of this process by as little as a few years would dramatically lower the percentage of elderly people living with cognitive or other brain-related deficits, he suggests. ■