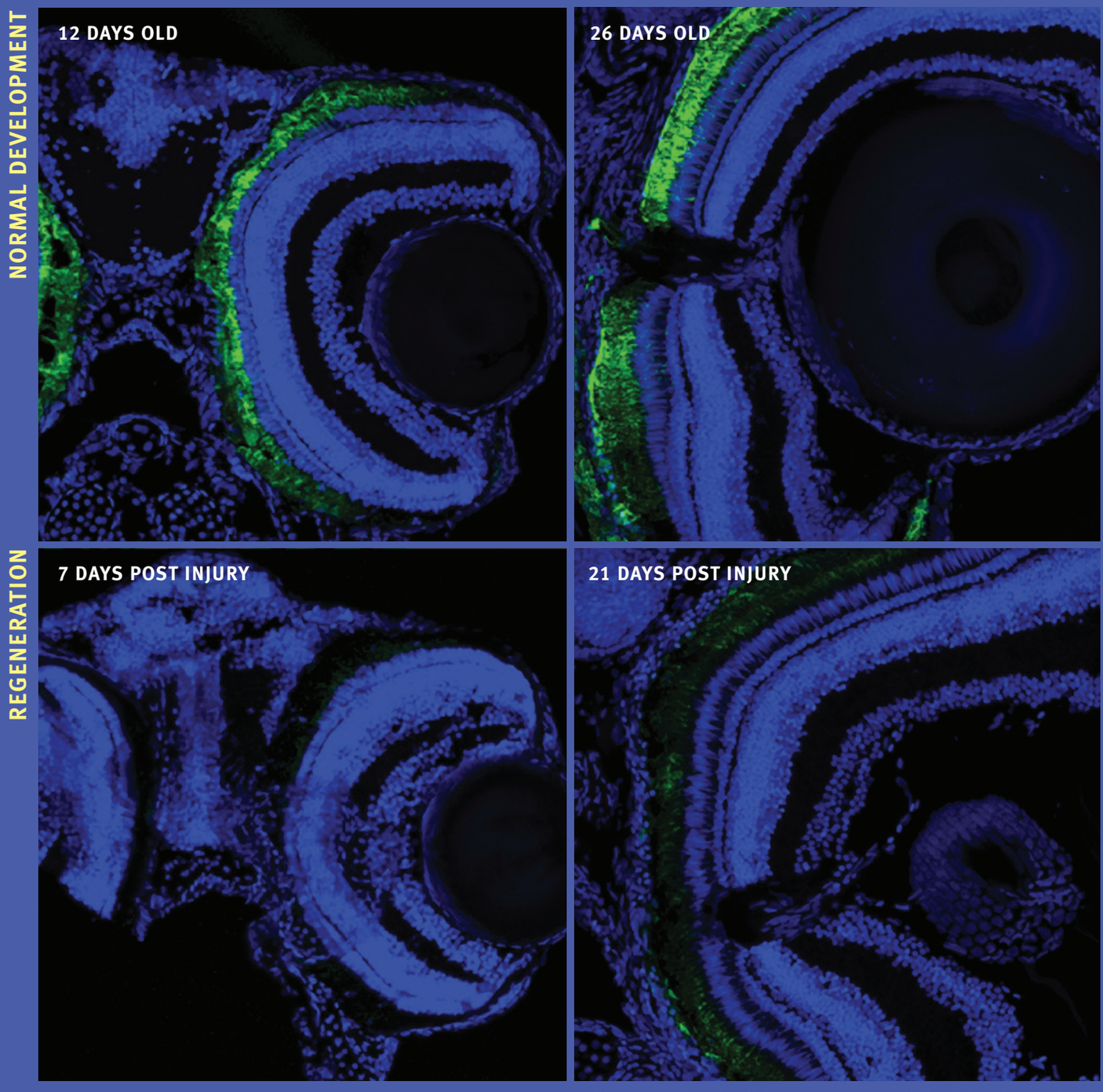


Unlike mammals, zebra fish can regenerate cells in their retinas and retinal pigmented epithelia (RPE) after injury. THIS PAGE: The top row shows normal development of the RPE in zebra fish (green). The bottom row depicts RPE regeneration in age-matched sibling larvae seven and 21 days after injury, when green RPE cells begin to reappear. OPPOSITE PAGE: At 44 days post injury, zooming in further, the zebra fish has fully recovered its RPE (green) and photoreceptors (aqua).

SEEING ANEW

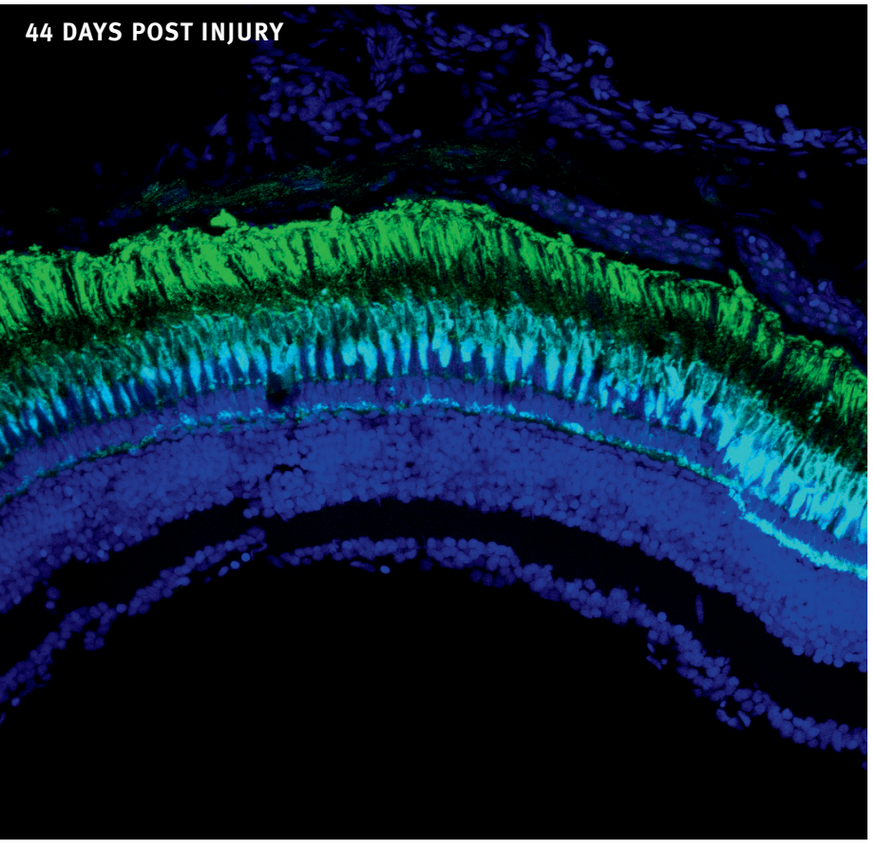
NAVIGATING RETINAL REGENERATION

BY KRISTIN BUNDY

"We FedExed all of our fish," says Jeffrey Gross as he steps out of a brightly lit room full of 11,000 thin, green tanks of tiny, translucent zebra fish.

In August, the 12,000-some fish embryos made the move from the University of Texas at Austin to their new home at the University of Pittsburgh School of Medicine, along with their handlers: four PhD students, two postdocs, and Gross, a PhD professor of ophthalmology and the E. Ronald Salvitti Professor of Ophthalmology Research.

Gross and his crew had been studying eye development and congenital eye diseases in Austin when Pitt enticed them with the opportunity to dive into the research they were most interested in—regenerative biology and its genetic and epigenetic mechanisms. For Gross, Pitt was an ideal setting: a med school that's both a clinical and basic science powerhouse. (The fact that the new digs are home to one of the largest zebra fish facilities in the country gave Pitt a fin up, too; Gross's fish joined some 11,000 tanks full in Biomedical Science Tower 3.)



COURTESY NICHOLAS HANOVICE, GROSS LABORATORY

Why zebra fish? Because they have a remarkable ability to regenerate their retinas, explains Gross. "And these are the tissues that are affected in a lot of blinding disorders."

In the zebra fish, Müller cells—the structural components of the retina that also play a role in homeostasis—regenerate in an elegant process. The cells recognize the injury, de-differentiate, determine which cell is needed, morph into progenitor cells, proliferate, and replace the injured cells.

In mammals, Müller cells recognize when an injury has occurred, but they get stuck in the process and don't de-differentiate or reinvent themselves before they proliferate. Instead, they build scar tissue.

Gross and his team plan on comparing mammalian Müller cells to those of zebra fish to see why they act differently. They are curious to learn whether gene expression influences the outcome.

"One idea is maybe the fish have evolved this amazing ability to [regenerate retinal cells], and there's a magic bullet that we don't have," he says. Another possibility: Perhaps mammals once had this ability but then lost it at some point in their evolution. If it's the latter, Gross suspects they may be able to find a way to circumvent the genes that are turned on or off and help mammalian Müller cells regenerate just as swimmingly as those of the zebra fish.

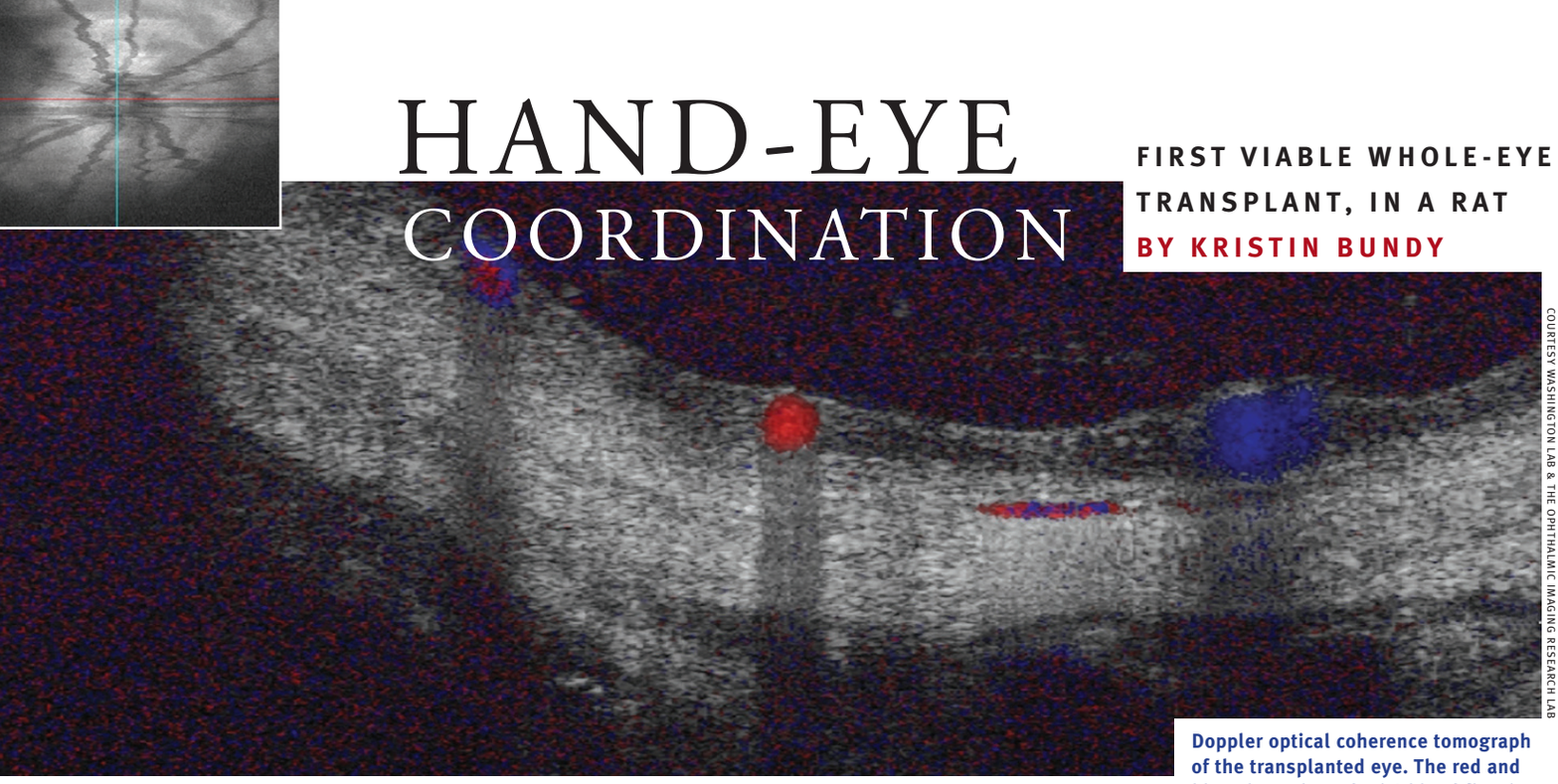
Gross is also the director of the Louis J. Fox Center for Vision Restoration—perhaps the world's first regenerative ophthalmology center—which is now funding his research. A collaborative, multidisciplinary venture between the UPMC Eye Center and Pitt's McGowan Institute for Regenerative Medicine, the center brings clinical and basic science faculty together to advance ocular regenerative medicine. "This really is something unique to Pitt," Gross notes.

"We have so much expertise in ophthalmology, on the research side [and] on the clinical side. The McGowan [Institute] is terrific for regenerative medicine. We have all of these resources here at Pitt that are supporting the Fox Center. . . . It's really a gem," says Gross. "Something that we can build off of to cure blindness."

HAND-EYE COORDINATION

FIRST VIABLE WHOLE-EYE TRANSPLANT, IN A RAT

BY KRISTIN BUNDY



COURTESY WASHINGTON LAB & THE OPHTHALMIC IMAGING RESEARCH LAB

Doppler optical coherence tomograph of the transplanted eye. The red and blue shown here depict blood flow in the vessels in real time.

"That's the rat right there!" says Kia Washington (Fel '08, Res '11, Fel '12), pointing to the scientific poster hanging in her office. "It inspires me. [And] I haven't really had a chance to decorate."

At first glance, it's a bit startling because, well, not too many people have photographs of rats on their walls. But look closer, and it's a marvel to behold—a white-furred, pink-eyed rodent that sees with an eye that was once not its own. This rat is the first viable orthotopic rodent eye transplantation model ever developed—a feat that was spotlighted at the Department of Defense's annual Military Health System Research Symposium in August—and Washington, an assistant professor of plastic surgery and associate director of the hand transplantation program at Pitt and UPMC, led the group that pulled it off.

Yes, you read that right: hand transplantation. Washington acknowledges, "People always ask me, 'Why is a hand surgeon doing research on eye transplantation?' And I will say it's kind of an evolution. You never know where things are going to take you."

A plastic and reconstructive surgeon by training, Washington completed a fellowship at Pitt's Thomas E. Starzl Transplantation Institute, where she built a functional face transplant model in the rat. This research, along with her clinical practice in microsurgery and plastic surgery (she has surgical privileges at both UPMC Presbyterian and the VA Pittsburgh Healthcare System) prepared her for eye transplantation research, she says.

It all came together when Vijay Gorantla, an MD/PhD associate professor of plastic surgery at Pitt, approached Washington two-and-a-half years ago about joining a multidisciplinary, multi-institutional collaboration to study eye transplantation. The team needed an animal model. "That's where my expertise of working on the face transplant model came in," explains Washington. "I said, 'Well, I can develop a model in the rat. We'll just add an eye.'"

She quips, but this is no easy endeavor. Transplanting an entire eye involves many different tissue types (as do hand transplantations). In addition to the eye itself, the procedure includes the transplantation of the extraocular muscles, subcutaneous tissue around the eye, vessels, the optic nerve, and sometimes skin, like the eyelid. They call this vascularized composite allotransplantation, or VCA.

Whole-eye transplantation, although not a new concept (it was first attempted in the late 1800s), hadn't been a viable option until just a few years ago, when Harvard's Larry Benowitz and colleagues discovered a way to coax the axons of the optic nerve to regenerate—impressive, considering the central nervous system doesn't typically regenerate easily.

Benowitz is a key member of the multi-institutional eye transplantation research group initiated by Gorantla. Joel Schuman, the former chair of ophthalmology at Pitt and former director of the Louis J. Fox Center for Vision Restoration, connected the Pitt team with Benowitz. Schuman also brought in Jeffrey Goldberg, chair of ophthalmology at Stanford, who is studying optic nerve neuroprotection and regeneration. Two PhD assistant professors of ophthalmology at Pitt—Kevin Chan and Michael Steketee—round out the group.

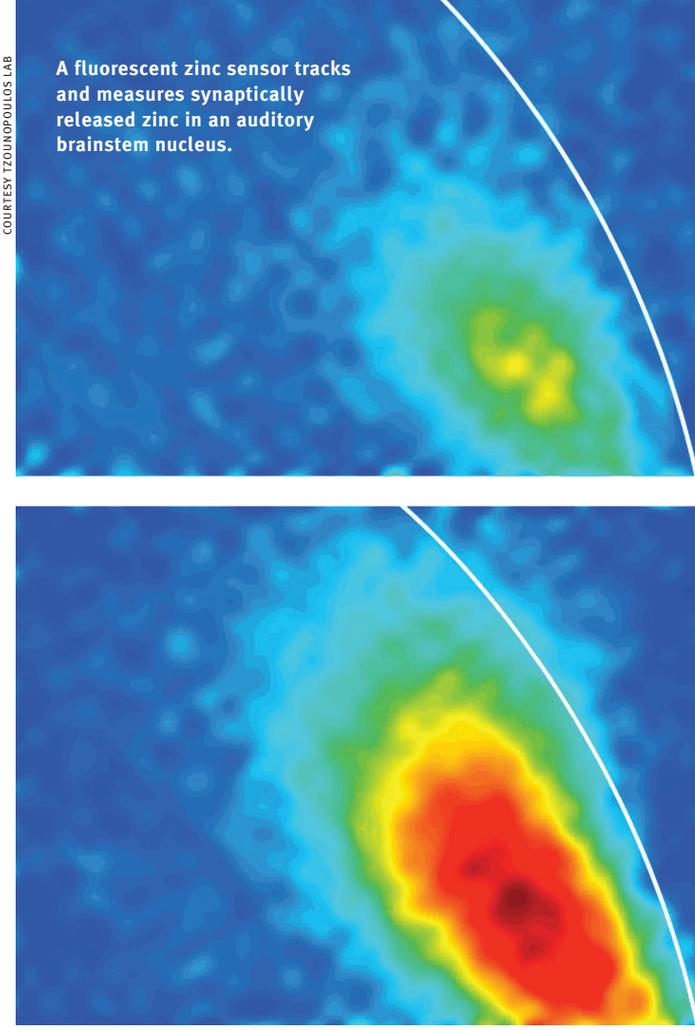
As Benowitz and Goldberg work on optic nerve regeneration, the Pitt people are optimizing and refining surgical procedures, transplanting the eye in Washington's rat model and in human cadavers. They will also focus (so to speak) on issues surrounding rejection, tissue viability, immunomodulation, and plasticity of the visual cortex.

"It's high-risk/high-reward research," notes Washington. "It could go nowhere, but if it goes somewhere, it would be incredible to actually be able to restore sight for somebody through this type of surgical intervention." She adds, "People are saying it's a pipe dream, a moon shot, which it is. But I think it will happen in my lifetime. Do I think it's going to happen five years from now? Probably not. Will it happen 20, 30 years from now? I think it's a high possibility." ■

THINK ZINC

FOR BRAIN BALANCE

BY ALLA KATSNELSON



COURTESY TZOUNOPOULOS LAB

A fluorescent zinc sensor tracks and measures synaptically released zinc in an auditory brainstem nucleus.

Our bodies are flooded with zinc—it is the most abundant mineral in the body after iron. It's thought to help power basic processes in the body, such as growth and immune function. Now, research from the University of Pittsburgh has found a specific and surprising role for the metal in regulating how neurons communicate; it probably figures heavily in neurodegeneration, learning, and memory.

Neurotransmitters—message-carrying chemicals—hang out at the branched endings of neurons in tiny sacs called synaptic vesicles. When it's time for a neuron to transmit a signal to the one next door, the vesicles dump their contents into the gap between the two cells. The neurotransmitters float across this synapse and dock at receptors on the other side.

Often, though, one key neurotransmitter doesn't make this crossing alone. For decades, scientists have observed that many vesicles containing glutamate—the main carrier of excitatory nerve signals in mammals—also contain free-floating zinc. Yet zinc's role in neurons was a long-standing mystery, because the tools available to track it weren't fast enough to follow its path. Researchers couldn't determine with certainty where it went or what its job was, says Thanos Tzounopoulos, a PhD associate professor of otolaryngology and neurobiology at Pitt, who also holds Pitt's Auditory Physiology Chair.

Tzounopoulos's group recently used new tools to reveal some answers: namely, that zinc is a neurotransmitter in its own right, and it holds a mighty vocation in a previously unknown pathway that the brain uses to fine-tune neural signaling. These findings were published in two *Proceedings of the National Academy of Sciences* articles last year.

Tzounopoulos, who studies the auditory system, had been curious about zinc's job description since reading some 15 years ago that brain areas involved in hearing have abundant amounts of the mineral. But he didn't get around to looking into zinc until arriving at Pitt in 2008. At a dinner following his job talk, he happened to sit next to Elias Aizenman, a PhD and Pitt professor of neurobiology who studies zinc's role in nerve-cell death. Conversation led to collaboration, which yielded a 2013 article showing that the mineral interferes with glutamate's excitatory signal. But technological limitations still hamstrung the prospect of determining its role more precisely.

Not long after he and Aizenman completed their study, Massachusetts Institute of Technology chemist Stephen Lippard heard Tzounopoulos give a talk about it. Intrigued, Lippard offered to develop a fluorescent sensor that could track zinc in a quantifiable way, as well as a chelator—a compound designed to intercept and capture zinc the instant it's released from the vesicles.

Tzounopoulos's group deployed these tools to look at a class of auditory neurons that receive signals from cells with zinc-rich synapses. They tracked zinc's effects on a particular type of glutamate receptor called the extrasynaptic NMDA receptor, positioned just outside the synapse. Tzounopoulos was particularly interested in what would happen at these receptors because neuroscientists are starting to believe that stimulating them can trigger cell death.

In their May 2015 study, the researchers showed that zinc diffuses into the synapse and surrounding region as a neurotransmitter and that it interferes with the extrasynaptic NMDA receptors. "This means that, in the same vesicle, you have an activator of the receptor—glutamate—and the brake for its activation—zinc," he explains.

By dampening such stimulation, Tzounopoulos adds, zinc could be having a neuroprotective effect. "These extrasynaptic receptors are extremely important [in] neurodegenerative diseases—when the balancing and fine-tuning of the receptors is out of whack, it can lead to neurodegeneration," he says. "They have to be kept in check."

The group's more recently published work demonstrates for the first time a wider role for zinc: The mineral serves as the gas and the brakes for another major type of glutamate receptor, called the AMPA receptor, involved in proper brain functioning. They found that zinc is corelased with glutamate in some areas of the neocortex (the most recently evolved part of the brain, which is often impaired in neurodegenerative diseases). This tag-teaming affects AMPA receptor response, which suggests that zinc has a fundamental role in modulating how strongly neurons connect to each other at any given time.

Further, it seems that zinc probably acts on other types of receptors besides glutamate receptors. "Now that we have the right tools," says Tzounopoulos, "we can explore what other partners of the synapse may be modulated by zinc." ■