

## INVESTIGATIONS

*Explorations and revelations taking place in the medical school*



In control mice, the pathway between the brain regions MNTB (medial nucleus of the trapezoid body) and LSO (lateral superior olive) is organized in an orderly fashion. Red neurons (as seen here) respond to high frequencies; purple and blue neurons respond to low frequencies. As the neurons' axons project from the MNTB (smaller oval on the left) to LSO (larger oval on the right), there is no, or little, intermixing of colors. For example, red ends on the left side of the LSO, and blue and purple end on the right. A pulse from the inner ear seems to ensure that the axons are organized appropriately for sound processing.

# TO YOUR OWN DRUM

## THE RHYTHM OF THE INNER EAR COACHES THE BRAIN TO HEAR

BY NANCY AVERETT

Many mammals are born deaf and don't begin hearing until weeks later. Until then, all's not exactly quiet, though—the inner ear is already pulsing with electrical activity. Researchers have long speculated about the function of this rhythm, which begins in the inner hair cells of the cochlea and travels along the auditory nerve to the brain. Now, University of Pittsburgh researchers have discovered that the precise pattern of this drumbeat is what tells the brain how to form the right connections for hearing and processing sounds.

Karl Kandler, a PhD professor of otolaryngology, neurobiology, and bioengineering at

hearing deficits. Their study, published in the May 2014 issue of *Neuron*, has implications for humans, whose inner-ear rhythms may occur in utero.

About 2 to 3 percent of children are born with good hearing but have difficulty interpreting the meaning of sounds—an impairment known as central auditory processing disorder (CAPD) that often accompanies disorders of speech, language, and learning. “In these children, something is wrong in the circuits in the brain that process sound,” Kandler says, adding that his team’s animal-model studies may be the first step to better understanding the biological cause of CAPD.

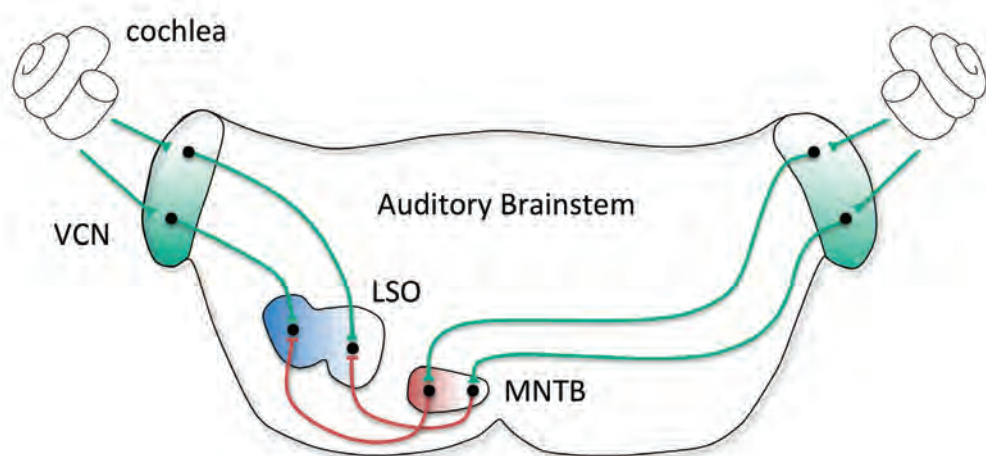
precise intervals in between. In his mutant mice that model CAPD, these patterns were slightly “blurred,” with impulses occurring more randomly. “That’s the only difference we found. Everything else we looked at in the ear was quite normal,” Kandler says. “We couldn’t believe it at first. . . . This little difference has huge consequences on the brain.”

The impulses spread along the auditory pathway into the brain, coaching it to set up networks for hearing. At first these networks, or neuronal maps, are not very precise. The neurons grow “exuberantly,” says Kandler, connecting to more targets than is necessary. Then, as the mammal matures, patterned neuronal activity prunes incorrect connections and strengthens correct ones.

Kandler looked at one specific area in the brain that is evolutionarily similar in rodents and humans: the inhibitory neuronal pathway, which runs from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO).

He found that as the mutant mice matured, the neuronal maps in this pathway did not go through as robust a refinement process as those in the control mice. The mutant mice had many more neuronal connections spread out over a wider area. “If the wrong rhythm drives them, the neurons cannot make a clear decision,” Kandler says. “They cannot really dedicate themselves to using one set of connections and cutting other ones. Instead they keep too many inputs going and keep them all in a weak state.”

Though the mutant mice could hear fine, they had trouble distinguishing different pitches. “What this study shows,” says Kandler, “is that small, very subtle deficiencies in the pattern of spontaneous activity in the inner ear before hearing onset can have very long-lasting consequences.” ■



Kandler’s group looked at an area of the brain important for, among other things, distinguishing low- and high-frequency sounds. This schematic shows neural pathways to the lateral superior olive (LSO). Red shows inhibitory pathways and green indicates excitatory ones.

Pitt, and colleagues studied a strain of mice with a genetic mutation that slightly tweaks the metronome. Kandler and his co-investigators found the resulting botched beat gave the mice subtle

Some background: Hair cells inside the cochlea emit a series of spontaneous electrical impulses. In Kandler’s control mice, those impulses occurred in predictable patterns with



A Pitt lab may have found a way to strengthen and heal cardiac tissue in infants born with weak hearts. Here we see a cardiomyocyte (red) undergoing cellular proliferation (green). Blue spots are cell nuclei.

COURTESY BALAKRISHNAN GANAPATHY

## HOW TO STRENGTHEN A TINY HEART, POUND FOR POUND

BY HEATHER BOERNER

# AN OUNCE OF REGENERATION

**T**he heart of a newborn is small but powerful—usually. For babies born with heart defects, though, the muscle can be marred by holes and malformed valves, or simply pump weakly. Surgery can close the holes and fix the valves.

But a weak heart? Medicine hasn't been able to do much about that. Beta blockers and ACE inhibitors, common treatments for adults, are ineffective in children. Worse, corrective surgeries can cause scar tissue that further limits the heart's power to pump. Left untreated, a weak heart can cause pediatric heart failure.

"This is where regeneration comes in," says Bernhard Kühn, an MD associate professor of pediatrics at Pitt and director of research in the Division of Pediatric Cardiology at Children's Hospital of Pittsburgh of UPMC. "If we can give these kids just half an ounce [more] heart muscle, that may potentially make a big difference for the duration and quality of their lives."

And it seems Kühn might have found a way to do that, at least in the lab. As reported in a *Science Translational Medicine* paper published in April, Kühn's team applied to injured mouse hearts and isolated human tissue a protein called recombinant growth factor neuregulin-1 (rNRG1). The protein is known

to stimulate cell growth in many organs and the nervous system. Kühn's team stimulated cell growth. This complex, multipart study not only found rNRG1 to be effective but also pinpointed the narrow window in which to target future clinical trials.

In a previous study, Kühn and his team found that people with healthy hearts can generate new heart cells until about age 20. But no one knew how long an infant with an unhealthy heart would be able to grow new heart cells. So they set out to answer that question, as well as the question of whether rNRG1 could kick-start the process.

After fine-tuning their own adaptation of an animal model of pediatric heart disease, the team studied the timing of rNRG1 administration. They had assumed starting at 4 days old would be plenty early—but not so. Frustrated, they started all over again, introducing the protein at birth.

Everything changed. "When we started seeing positive results," says Balakrishnan Ganapathy, a lab manager and research technologist at Children's, "we realized we were on to something really big."

Among rodents that received rNRG1 at birth, only one in 10 still had a scar across the entire thickness of the heart wall when

they were examined later (compared to 75 percent of the hearts that received rNRG1 at 4 days old). That is to say, rNRG1 seems to generate cell growth, which can repair heart damage and build a stronger muscle. What's more, rNRG1 seems to protect healthy heart tissue from dying.

"We do now have, in the data, some indication that the scar is now pumping [blood]," says Kühn.

At the same time, the lab was studying human heart tissue from infants who'd undergone heart surgery. They found that in the dish, the heart cells stopped proliferating in patients older than 6 months of age. And for those infants who did exhibit cell growth, it was slow.

But when they applied rNRG1 to the cells?

"The surprising thing is that the heart-muscle pieces actually liked it," says Kühn. "They had striations, so their molecular motors were visible with the microscope. The striations were still parallel, meaning when they contracted, they all pulled in synchronicity. The electrical connections were still present."

Kühn and his team are hopeful that the same treatment might be effective in clinical trials that target children from birth to 6 months of age. Ganapathy, meanwhile, has begun studying the safety of rNRG1 administration in rodents. So far, he's seen no serious side effects.

In this era of ever-improving neonatal medicine and surgery, the question is less often, *Can we save this baby?* says Kühn.

"The question is," he says, "*How will this person live when he or she is 10, 20, 30, 40, 50, 60, 70, even 80 years old?*" ■





OXYGEN CAN  
BRING DOWN TUMOR  
DEFLECTOR SHIELDS  
BY CHERYL ALKON

# FIRE AT WILL!

**I**n the *Star Trek* universe, when the *USS Enterprise* encounters a dreaded class of warship called the Bird of Prey, it's a nail-biter: Not only can the attacking Klingon vessel raise its deflector shields—standard-issue 23rd century technology that protects starships from enemy fire—but it can also activate a unique “cloaking” device, a force field that renders the Bird of Prey virtually invisible.

Cancer works the same way, says Edwin K. Jackson, a PhD professor of pharmacology and chemical biology at the University of Pittsburgh. Tumors “try to cloak and shield themselves so they can't be seen or attacked by the immune system,” releasing chemicals that keep them from being taken out. They “blind” the immune cells and “put them to sleep” to prevent attack.

But Jackson—along with the study's principal investigator, Michail Sitkovsky, a PhD and director of the New England Inflammation and Tissue Protection Institute at Northeastern University, and 16 coauthors from Northeastern, the University of Miami, and Boston's Brigham and Women's Hospital—has found what might be the key to deactivating tumors' deflector shields: oxygen.

Typically, tumors grow fast, and as they expand, their blood-supply needs increase, Jackson explains. An inadequate blood supply leads to a low-oxygen environment, which stimulates the production of adenosine, a molecule

that helps to suppress the immune system so that it doesn't recognize the tumor. And even if it does, the immune system's killing mechanisms are inactivated.

As reported in the March 4 issue of the journal *Science Translational Medicine*, Jackson, Sitkovsky, and colleagues discovered this by studying mice with breast cancer, melanoma, and a connective-tissue cancer called fibrosarcoma, giving them air with 40 to 60 percent oxygen (ordinary air is only about 21 percent). The team found that the increased oxygen levels caused the tumors in some mice to either decrease in size or disappear altogether. Jackson notes that the technique would likely work on any solid tumor regardless of the location in the body.

But oxygen alone isn't the trick to shrinking tumors in people, Jackson says. The idea is to combine oxygen with existing treatments, such as immunotherapy, to make them more effective. “We have a lot of work to do, but we have a very strong direction,” he adds.

Jackson and Sitkovsky's next steps: identify all the adenosine-like molecules in a tumor—chemicals that are collectively known as the purine metabolome—then learn how much of each compound exists. Then they'll determine their effects on the immune system. “We want to look at the purine metabolome in cell culture, then in animals, and then see if we

can reprogram it from an immunosuppressive setting to one that encourages immune cells to move in,” says Jackson.

He and Theresa Whiteside, a PhD professor of pathology, immunology, and otolaryngology at Pitt, are collaborating to investigate how immunosuppressive regulatory T cells and regulatory B cells protect tumors against immune attack, and whether and how adenosine is involved in these processes.

“I think we could get clinical trials going in the next year,” says Jackson. “As with any technology, I think as we learn more at the basic level, we can fine-tune clinical trials.”

Oxygen therapy has been tried before. The reason it hasn't worked, says Jackson, is because it wasn't administered correctly. His team's recipe: inhaled oxygen at 40 to 60 percent (depending on the patient's tolerance for oxygen), 24 hours a day, for several weeks—and, importantly, beginning the oxygen therapy at the same time as the start of a course of immunotherapy.

Challenges lie ahead, such as determining exactly how many weeks oxygen therapy should be used, developing a simple, patient-friendly device for it, and optimizing it by combining it with adenosine receptor blockers.

“The nice thing is that oxygen is available everywhere,” Jackson says. ■