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STATIC

TINNITUS IS ONE WAY OUR BRAINS LEARN AT THE CELLULAR LEVEL

BY ELAINE VITONE

The Ebers Papyrus, one of the oldest surviving medical texts, describes a mysterious condition the ancient Egyptians called "bewitched ears." Throughout the centuries, its characteristic ringing, buzzing, hissing, static, or other noises have cursed such notables as Charles Darwin and Ludwig van Beethoven—some historians speculate they're what drove Vincent van Gogh to cut off his own ear. Today, 10 to 15 percent of the population experiences tinnitus, as it's now called, chiefly older people and those who've been exposed to loud sounds, from machinists to musicians. For some, it's an occasional nuisance, but for up to 10 percent of sufferers, it's debilitating. With its consequences of fatigue, cognitive impairment, and depression, tinnitus is finally getting its due attention. It's the most prevalent service-associated disability for veterans of the wars in Iraq and Afghanistan.

There is no surefire cure, though some find relief in masking their tinnitus with other sounds. "I don't know what silence sounds like anymore," said Will.i.am of the Black Eyed Peas in an interview with *The Sun* last winter, in which he repeatedly wiggled his finger in his ear and shook his head. "Music is the only thing which eases my pain. . . . There's always a beep there, every day, all day. Like now. I don't know exactly how long I've had this, but it's gradually gotten worse."

In spite of their prevalence, historically, these phantom sounds have remained just that. As recently as 20 years ago, we were looking for their source in the wrong place: the ear. (Tinnitus *is* associated with hearing loss, after all.) Then came the reports that people whose sense of hearing had been completely dismantled—cancer-surgery patients whose auditory nerves had been cut—also suffered from tinnitus. This was the ultimate proof that it wasn't the ears that had been "bewitched" at all; it was the brain.

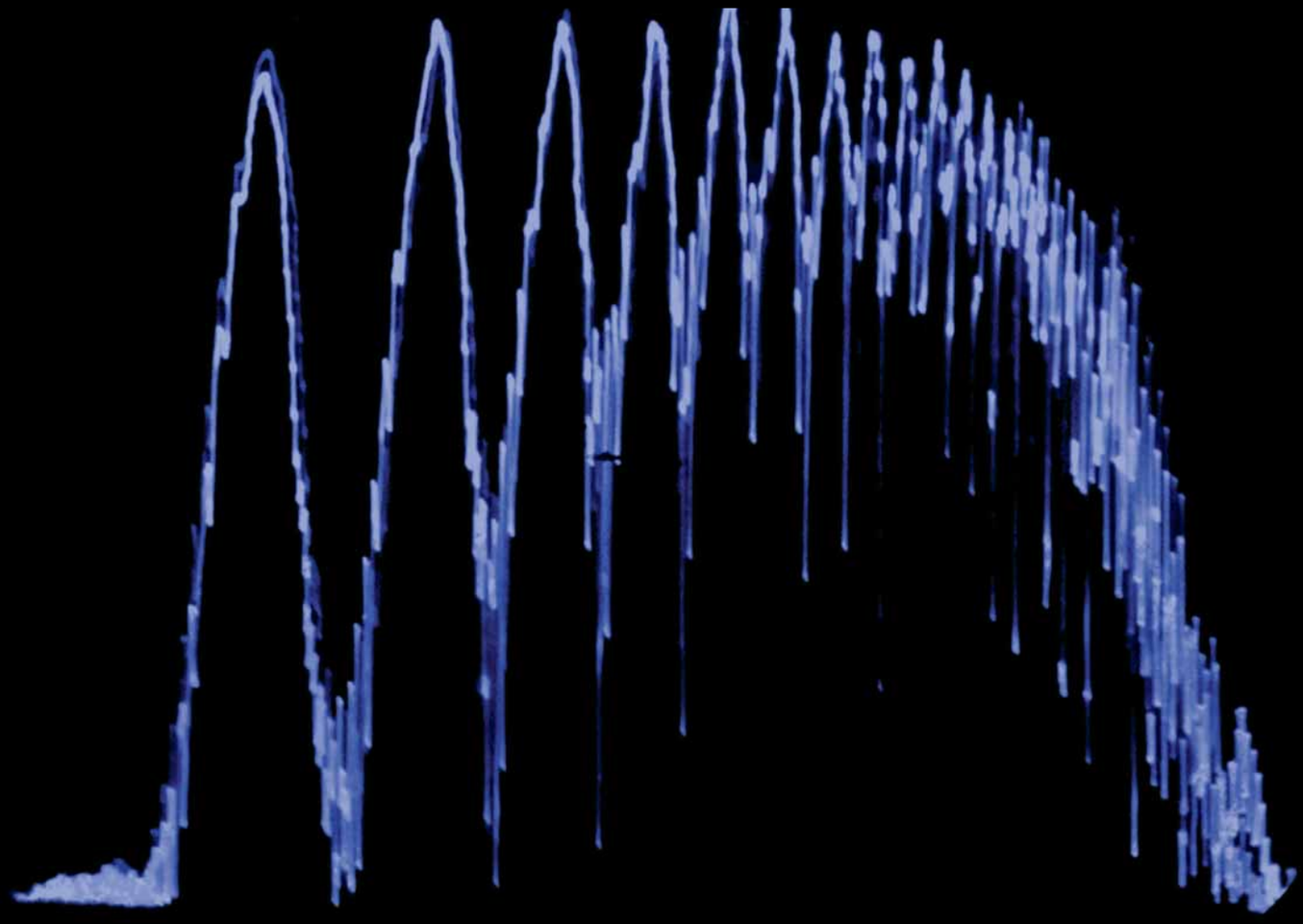
Thanos Tzounopoulos, a PhD assistant professor of otolaryngology, recently became the first to watch tinnitus in action at the cellular level, finally uncovering exactly how some brains turn on themselves in this way. He's learned that tinnitus is a betrayal of our biology that's rooted in the very strengths that have enabled humanity's success: memory, learning, and adaptability. Ironically, the cellular savvy that made Beethoven arguably the greatest composer the world has ever known also drove him to despair, his mind caught in a loop of "rushing, roaring sounds."

Tzounopoulos speaks with a Greek accent (he hails from Athens) and an easy laugh. In his jeans, Converse All Stars, and sideburns, he looks a bit more like a guy on his way to a rock concert than an international expert in the molecular aftermath of exposure to deafening decibels. The young investigator has been pushed to the main

COURTESY T. TZOUNOPOULOS

ABOVE: Thanos Tzounopoulos found that the brain centers that govern our senses are plastic just like those that enable learning and memory. RIGHT: Principal neurons of the dorsal cochlear nucleus—the first nucleus that ushers sound signals into the brain. This area is more active in mice with tinnitus than in healthy mice.

COURTESY M. RUBIO



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When loud sounds damage the hairs in the ear that turn sound-wave vibrations (such as these from a musical instrument) into a signal for the brain, hearing is lost. Yet people with tinnitus (associated with hearing loss), find loud sounds unnerving. Tzounopoulos saw this hypersensitivity as a clue that their brains had adapted, trying to make up for what their bodies now lack.

stage in recent months, finding his decade-long study of sensory processing suddenly of interest to the likes of *The Wall Street Journal* and NPR. "Whenever my research has direct implications for disease, it's as good as it gets for me," he says.

Tzounopoulos cut his research teeth studying the hippocampus (the brain center for memory and learning) as a postdoc in the lab of Roger Nicholl and Robert Malenka, trailblazers in the field of brain plasticity at the University of California, San Francisco. It was the 1990s, and the field was ripe with promise. Experts were buzzing over something called long-term potentiation (LTP), a phenomenon in which stimulating individual synapses causes the strength of their responses to increase. "It's a kind of training," Tzounopoulos says. Though LTP was first described in the late 1960s, its exact molecular mechanisms had remained elusive, but now, they were finally coming into focus. Scientists were becoming more and more confident that LTP was exactly what they'd hoped: a mechanisms of plasticity. Learning, at the cellular level.

A few years later, when Tzounopoulos began his second postdoc, he was eager to carve out his own niche. He knew he was going to stick with memory and learning—he wanted very much to understand the mechanisms of this training. But he decided it would be best to center his studies further upstream from the hippocampus, which gets involved very late in the process of learning. He settled on the brain's sensory processing—specifically, the very first structure in the auditory-processing chain, the auditory brain stem. "Because there you know what the information is about: sound."

At the time, no one believed the structure was plastic. The hearing portion of the brain was just the messenger, everyone assumed—it leaves the fancy work to pros like the hippocampus.

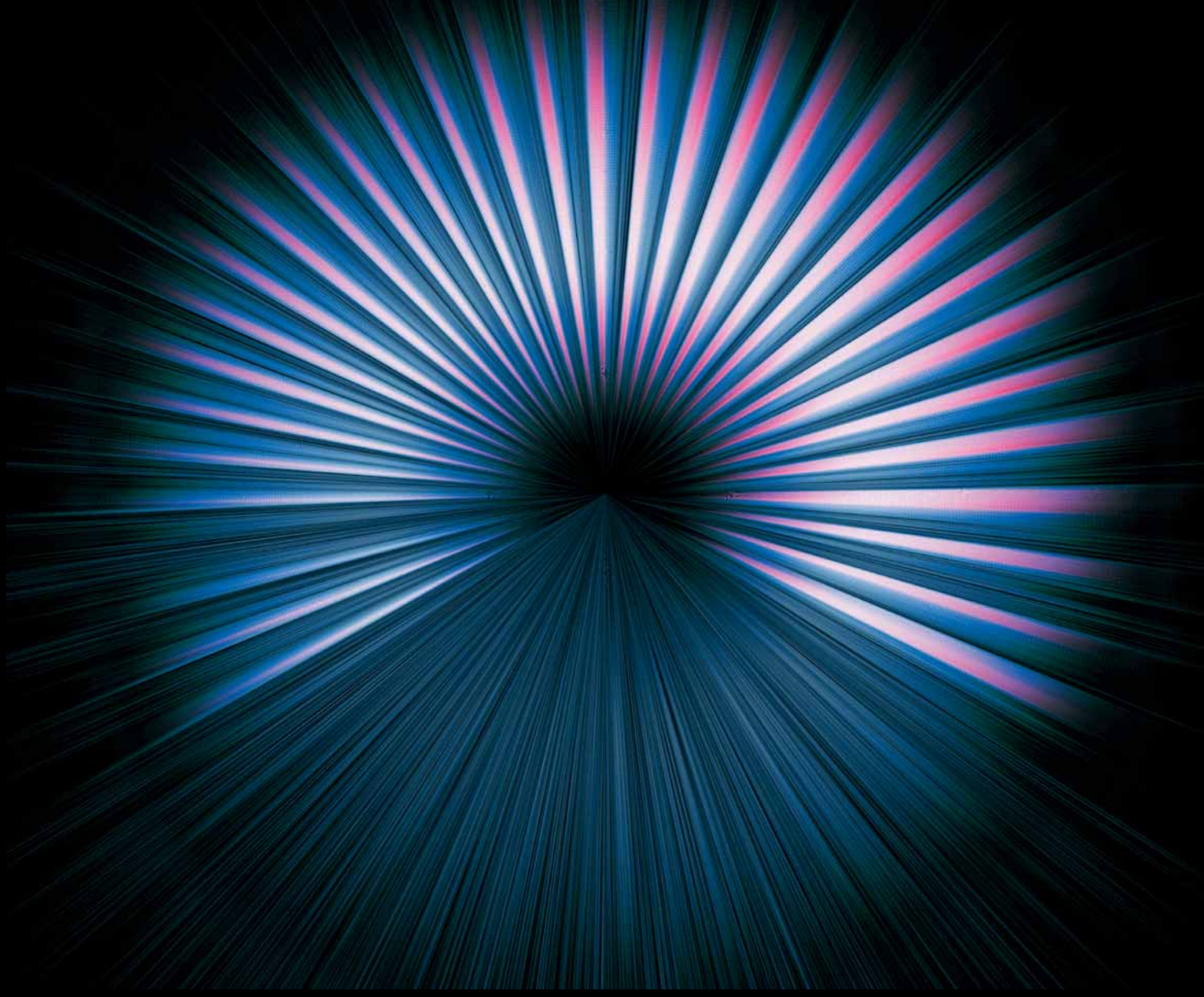
But Tzounopoulos noticed that the auditory brain stem had the same sort of wiring and organization that characterizes the cerebellum, the site of motor coordination.

"It was a gamble," he admits. But it worked. He stimulated individual synapses in the auditory brain stem and found LTP there, too. In 2009, he published a review of his findings in *Neuron* at the journal's invitation.

Next, as a great many scientists before him, Tzounopoulos sought to further understand healthy brain functioning by observing what happens when it's not so healthy. In the '50s, scientists discovered what the hippocampus was for by talking to a brain-surgery patient who was incapable of making new memories, Tzounopoulos recalled. So, about four years ago, he decided to study plasticity in the auditory brain stem by observing what happens when plasticity mechanisms misstep and the plasticity of the brain bends out of control.

As far as he could tell, that's what tinnitus was.

When hearing is damaged, the central nervous system must sense it, he figured. Wouldn't it stand to reason that the brain would want to maintain a certain level of activity? Could it be that it was trying to fill the silence on its own? It had been well documented in imaging studies that in people with tinnitus, the auditory circuitry's response to sound is far more pronounced. The system



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is fundamentally different in people with the disorder—perhaps because it's been trained to be, he thought. Just as auditory synapses in a petri dish can be trained to rev up. Just as a healthy hippocampus can be conditioned to recall the lyrics to "Ode to Joy."

"Nature, in a sense, is conservative," he says. "Once it's found solutions, it keeps using these solutions."

Sound travels in waves, vibrations that are picked up by the hairs of the inner ear. These hairs convert the vibrations to a chemical signal, then pass them to the brain through the auditory nerve. Inside, the auditory nerve stimulates the dorsal cochlear nucleus (DCN), the first nucleus in the auditory brain stem. The portal to the hearing brain.

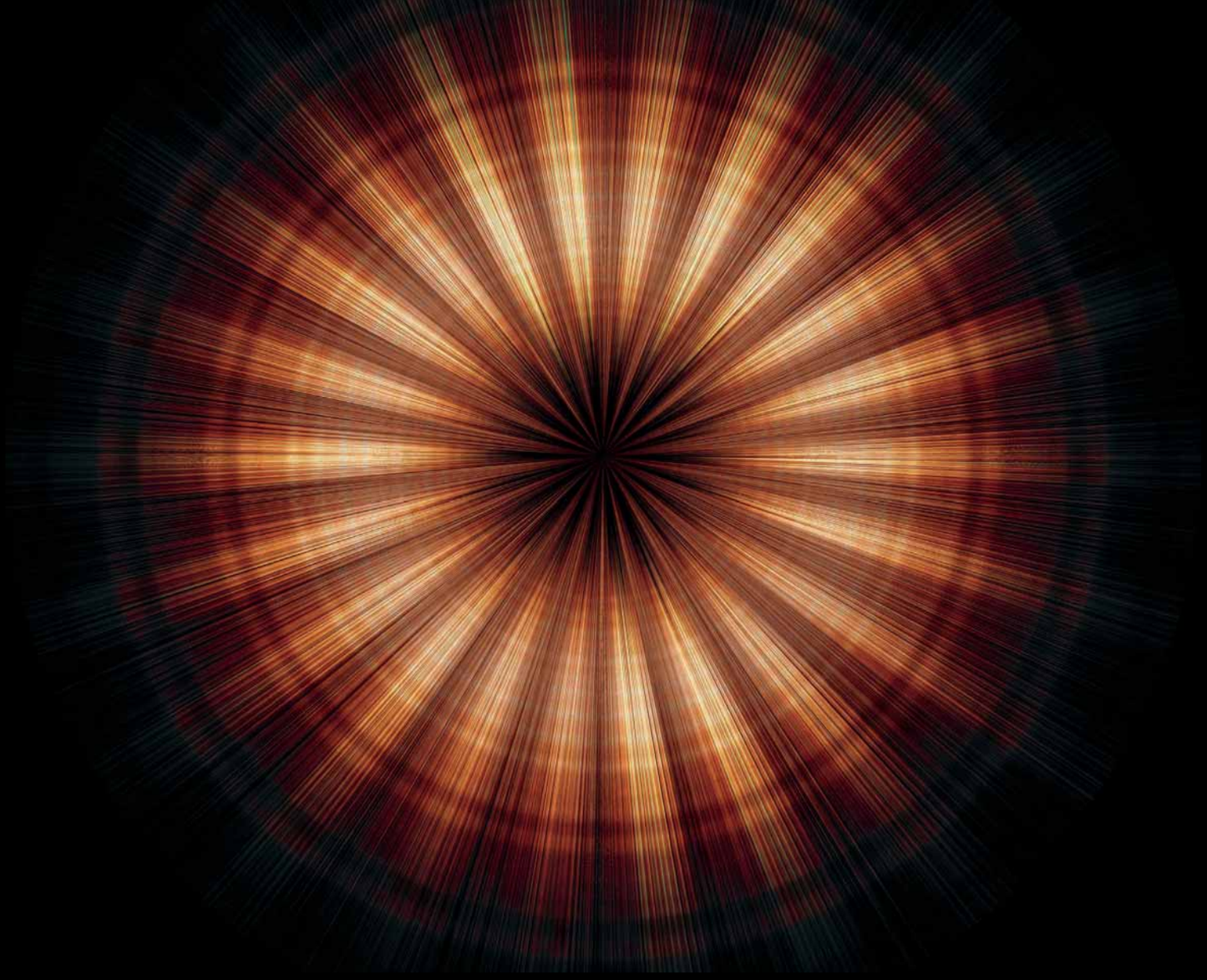
Once inside, the signal becomes part of the constant balancing act that is synaptic function: Excitatory forces move to increase signal activity on the one hand, and inhibitory forces decrease activity on the other. With the stimulation of a sound, the excitatory force increases, and a neuron fires. The process continues duplicating along the neurological chain, and, eventually, the person perceives sound. But in tinnitus, the neurons fire without the sound, which Tzounopoulos figured could be happening for one of three reasons: Either they're exposed to more excitatory force, less inhibitory force, or both.

To find out which, Tzounopoulos' team used a mouse model of tinnitus. Under sedation, the rodents were exposed to sounds about as loud as an ambulance siren (116 decibels) for 45 minutes. Weeks later, the team confirmed which mice had tinnitus by conducting startle experiments: The team played for the mice a 70-decibel tone, then interrupted it, and then resumed it before sounding a much louder pulse. The healthy mice perceived the gap and jerked with surprise; in the mice with tinnitus, however, their internal noise masked the silence in that gap.

The team then studied brain slices of the mice, watching how the synapses responded when they tweaked the balance of excitatory and inhibitory forces. As it turned out, amping up the former had no effect. But blocking inhibition did. Specifically, they found, deficiency in the inhibitory neurotransmitter GABA is the culprit in tinnitus.

On a fall afternoon in 2011, in his office in Biomedical Science Tower 3, Tzounopoulos cracks open his laptop and pulls up two movie files side-by-side on the screen: one with an outline of a dorsal cochlear nucleus of a mouse with tinnitus, and the other with the DCN of a healthy mouse. The cells are rendered with flavoprotein autofluorescence (FA), a technology he mastered while a fellow at the Marine Biological Laboratory in Woods Hole, Mass. Within the mitochondria—the powerhouse of the cell—are proteins called flavines, which fluoresce when oxidized with use. The brighter the glow, the stronger the activity.

Tzounopoulos clicks PLAY on the healthy mouse reel, and a small portion of it flashes red, then dissipates into orange and yellow. "But if you do the same thing in tinnitus,



Some tinnitus sufferers experience their phantom frequencies as buzzing or chirping sounds, like the song of a cricket (opposite page, converted mathematically into graph form) or cicada (above). Author and radio personality Garrison Keillor finds that cicada serenades mask his tinnitus perfectly. *Medicine has no remedy*, he wrote in 2007. *My only alternative, I guess, is to wander the planet in search of cicadas.*

look what's going to happen," he says, hitting PLAY on the diseased mouse model's movie. This is no small, localized response. "The whole area lights up." (Tzounopoulos published his findings in *Proceedings of the National Academy of Sciences* in 2011; he presented these movies at the international Tinnitus Research Initiative Conference last summer in Niagara Falls. See our Web Extras at pittmed.health.pitt.edu.)

Tzounopoulos is excited about the road ahead as he continues to sort out the story of tinnitus: What are the intrinsic, molecular properties of cells that dictate how adaptable they will eventually become? What exactly causes the decrease in GABA? Is less of it released? Are there fewer GABA receptors in play? Or has the circuitry reorganized itself so that there are fewer GABA-simpatico neurons? And just what exactly separates those who develop tinnitus from those who don't?

And then, of course, there are the therapeutic possibilities. Based on some very preliminary data, Tzounopoulos hopes he has a lead. In recent years, his team discovered that a certain neuromodulatory system—the cannabinoid system—is central to all forms of brain plasticity, sensory and otherwise. Further: "This system is very dominant in the auditory brain stem, and it mediates these ups and downs of synaptic strength," he says.

It's a target that's worked well in the past. The cannabinoid system—so named because its receptors are what cannabis binds to when the brain is exposed to marijuana—has also been linked to numerous physiological processes, including appetite, mood, and the sensation of pain. Hence, medical marijuana has been useful in treating chronic pain, which is now considered an apt parallel to tinnitus. Chronic pain is a similar story of the body betraying itself. "There is some peripheral damage that leads to a central response," Tzounopoulos says. "And this response then goes out of control."

In addition to chronic pain, mechanisms of plasticity have been found to be a driving force in addiction, Tzounopoulos points out.

"It's the same mechanisms that wire the reward systems. You get a reward—you feel good for doing something, so you want to do more of it. Constant abuse messes up these mechanisms. It's this learning route induced to an extreme. The addicted brain gets stuck in that state."

Nature is conservative, Tzounopoulos has noted. Rather than coming up with a whole new song and dance for every occasion, it tends to run through a familiar playlist. ■

WATCH FOR THE PITTMEDCAST THIS JANUARY ON ZINIO.