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MUSIC

VOCALIZATION VERSUS SONG

THE ORIGINS OF HUMAN MUSICALITY

THE NEUROBIOLOGY OF MUSIC THERAPY

THE PRODUCTION AND NEURAL PROCESSING OF MUSICAL SOUNDS, FROM BIRDSONG TO HUMAN SYMPHONIES

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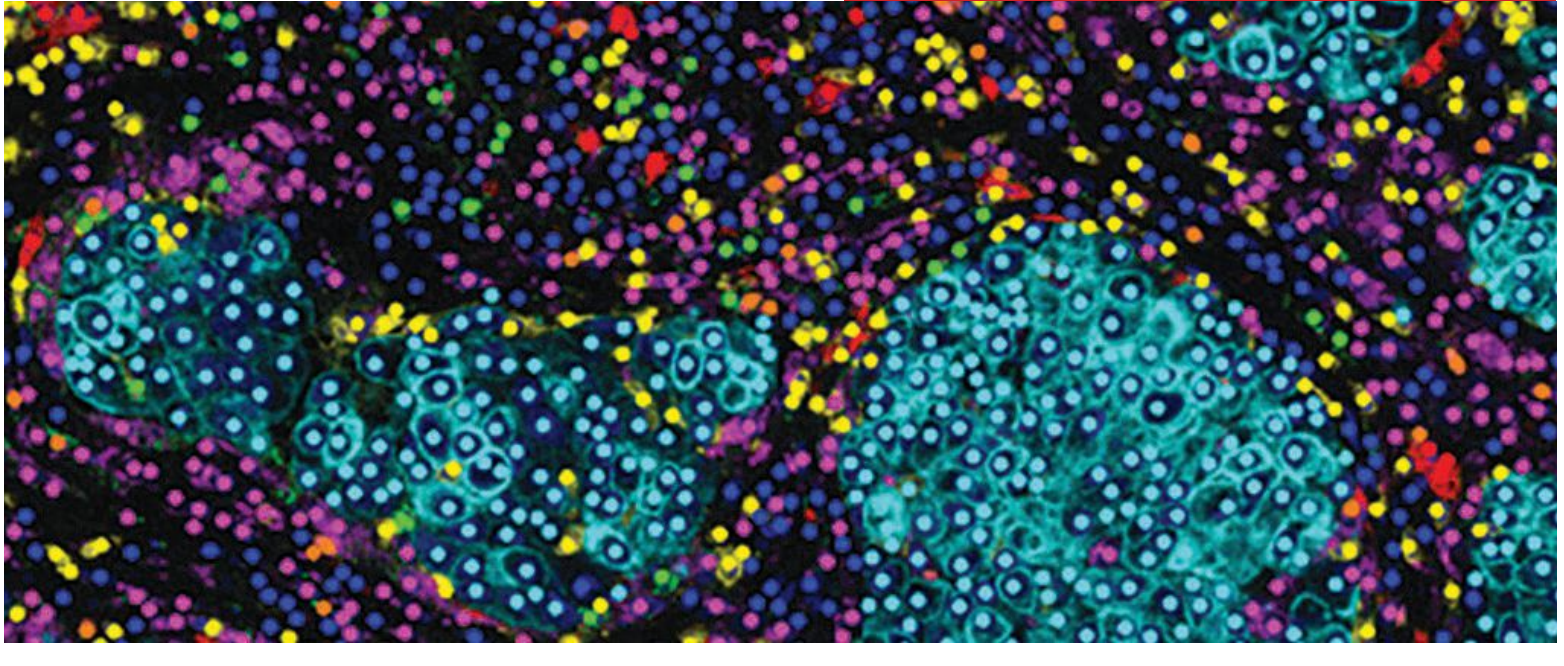

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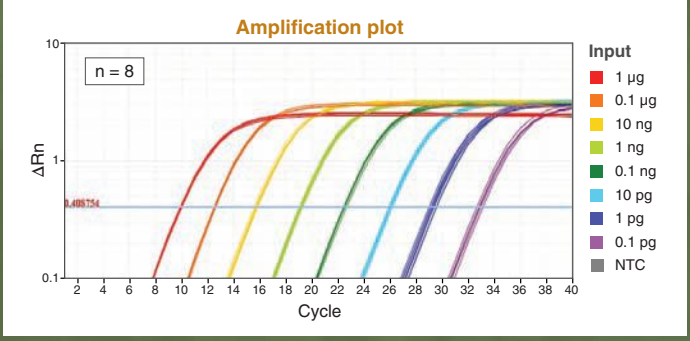

RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart® Reverse Transcriptase. NTC = non-template control

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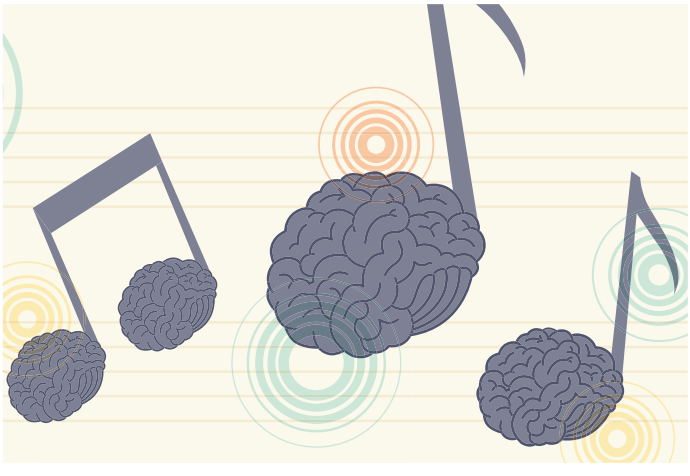

Features

ON THE COVER: ILLUSTRATION BY LYNN SCURFIELD

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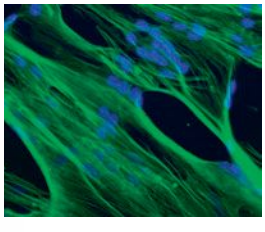

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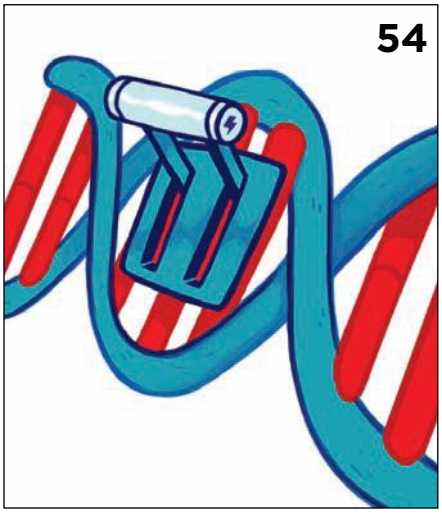

CARLA Y. BOE; © FRANK ROGOZIENSKI; © WHITNEY SALGADO

CORRECTION:
In "Parent Trap" (*The Scientist*, January 2017), the article stated that patterns of genetic imprinting vary between tissues and developmental stages. In fact, imprinting patterns are consistent across tissues and development; what varies is how cell types read those imprinting marks and whether they express the imprinted alleles differently.

The Scientist regrets the error.

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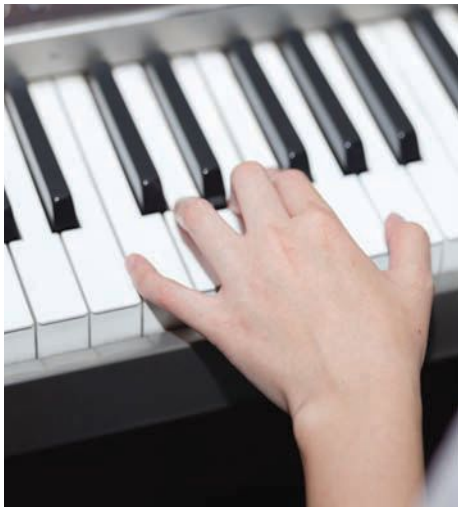

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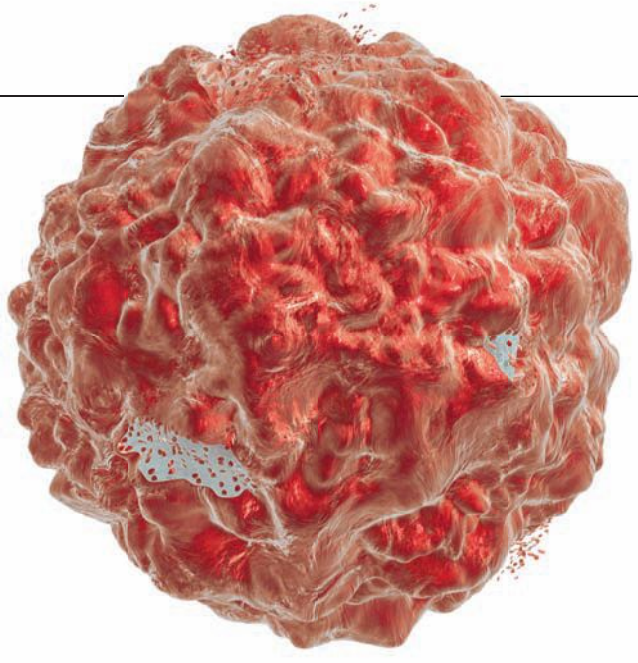

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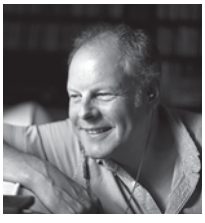
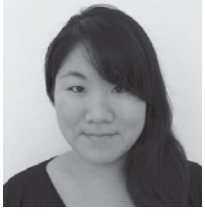
Contributors

Elizabeth Stegemöller began piano lessons at age five and played the French horn in her middle school band as well as in high school, where she also sang in the choir. She had no plans to become a musician, however. Instead, she left for college at the University of Missouri wanting to become a physician, but changed her mind once she started taking courses. "It just wasn't the right fit for me," she recalls. She declared a major in biology, but after a good friend introduced her to the concept of music therapy, she decided to add it as a second major. "I didn't like to perform all that much, so using [music] to help other people was right in line with what I thought was most interesting," she says. After spending several years as a music therapist, Stegemöller went on to pursue a PhD in neuroscience at Northwestern University. "There are people who have a stroke and can't speak but can sing, or people who can walk with music but freeze without, and I wanted to figure out how that works," she says. Currently, she is an associate professor at Iowa State University, where she has combined her two passions: music and the brain. In "Music Therapy" on page 42, Stegemöller examines the neuronal mechanisms associated with successful music therapy treatment.



Tim Falconer has been a journalist since 1983, and started off writing about computers for a magazine called *InfoAge*. He became a freelancer in 1985 and in 2001 published his first book, *Watchdogs and Gadflies: Activism from Marginal to Mainstream*, which was about political activism in Canada. He studied mining engineering and English literature at McGill University, and journalism at Carleton University. Falconer is passionate about music, but has never been able to be a musician. "I always thought I was tone deaf, but I didn't know what that meant," he says. He found out in 2011, after visiting an auditory neuroscience lab at the University of Montreal. "After my second singing lesson, I went down to Isabelle Peretz's lab in Montreal and she gave me the bad news that I was amusic," he recalls. Unlike most others with his condition, an inability to process pitch that is commonly known as tone deafness, Falconer loves music. In "Caterwauling for Science" (page 60), he writes about his experience and the science behind amusia.



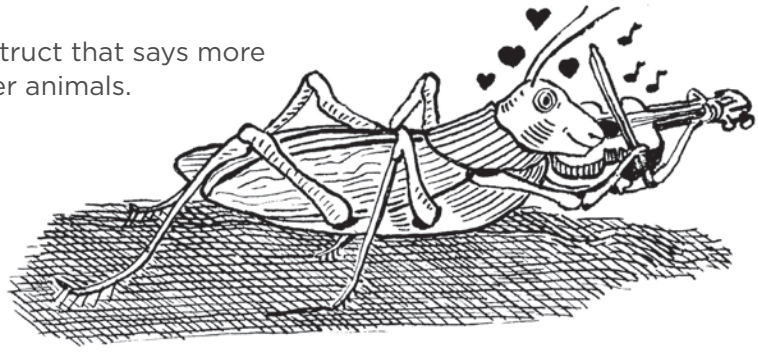
In high school in Toronto, Ontario, **Diana Kwon** took all the basic sciences—chemistry, physics, and biology—and headed off to undergrad at Queen's University in Kingston with the intent of studying to become a doctor. "I realized I liked science itself more than the medical side of it," she recalls, and she changed her focus to research. At Queen's, she worked with human volunteers, studying their decision-making behavior. In 2012, Kwon enrolled as a master's student at McGill University in Montreal, where she used functional MRI scans to study how episodic memory changes as people age. But again, she found herself on the wrong path. "Most of my time was spent doing statistical analyses, going through massive data sets," she recalls. "It was not fun for me." As Kwon began to think about science journalism as a possible career, she started writing for a student blog, then the McGill newspaper. In 2015, she interned at Fermilab, writing about particle physics, and then at *Scientific American MIND*. Last year, Kwon moved to Berlin and worked as a full-time freelancer for a year before choosing to join *The Scientist* as an intern. "I felt like I needed more experience," she says. "*The Scientist* offered something different, where I could write about all types of life science and continue to hone my skills."

You can read Kwon's contributions to the March issue in the Notebook (page 15) and Literature (page 49) sections.

Song of Ourselves

"Nature's melodies" may be a human construct that says more about us than about the musicality of other animals.

BY BOB GRANT



In the wee hours of the morning earlier this year, my restless wife lay in bed and groaned: "That cricket must die. Now." The amorous insect, perched very near our bedroom window, had been steadily chirping since sundown the previous evening. While well and good for his chances of securing a mate, the animal's volubility was endangering the harmony of my own marital union.

Nevertheless, we let him sing. And as I listened to the six-legged crooner, I was reminded that music is in the ear of the beholder. Or more precisely, in the beholder's brain.

Thinkers have waxed poetic about the musical qualities of birdsong for centuries. "And hark! the Nightingale begins its song, / 'Most musical, most melancholy' bird!" wrote Samuel Taylor Coleridge in 1798. A few decades later, Percy Bysshe Shelley celebrated the skylark: "Hail to thee, blithe Spirit! / Bird thou never wert, / That from Heaven, or near it, / Pourest thy full heart / In profuse strains of unpremeditated art."

Even Charles Darwin was guilty of romanticizing music in nature. "Musical notes and rhythm were first acquired by the male or female progenitors of mankind for the sake of charming the opposite sex," he wrote in *The Descent of Man*.

But are the refrains of nature—from melodious birdsong to the emergent rhythmicity of insect calls to Darwin's hypothesized mate-charming proto-serenade—actually "music"? Or is it we humans who bring that label and all its cultural baggage to the party?

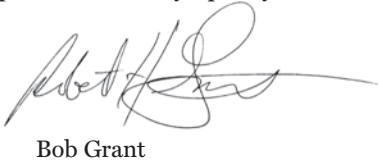
Science tells us that our brains are expert pattern generators. So adept is the human brain at creating order out of disorder that it can find religious figures in burnt toast and dragons in a cloudy sky. Perhaps, then, it is also finding music in the sounds of nature.

As is the hallmark of interesting lines of scientific inquiry, research into the biology of music has precipitated more questions than answers. I can report that whether nonhuman animals produce "music" is far from settled. Some researchers we interviewed for this special issue on music suggested parallels between a singing male bird and a rock star wailing away before adoring fans. Others rejected the idea that human music and the vocalizations of birds or bats or whales have much to do with each other at all. They framed the vocalizations and instrumentations of nonhuman animals as dispassionate, almost automatic utterances, honed by evolution to efficiently communicate specific messages to their intended audiences.

While I know well the dangers of suggesting that specific behaviors are uniquely human, I also realize that some of our eccentricities do set us apart from the rest of the animal kingdom; wearing pants and watching TV come to mind. So are we merely one player in a vast animal orchestra? Or are we alone in producing and listening to music for sheer enjoyment? I tend to agree with the latter, but remain open, as ever, to evidence-based shifts in my thinking.

Of course, all of this conceptual wrangling means very little to our friend the cricket. Although he didn't return to our window for an encore after so annoying my better half, I'm positive his own biology compelled him to chirp the night away elsewhere, blissfully unquestioning of his innate drive to do so.

This is not to say that I begrudge Coleridge or Shelley or Darwin the pleasure of finding beauty or charm in the chorusing of birds or the thrumming rhythms of water and wind. I delight in some of the very same patterns, not to mention the musicality of an overloaded washing machine or a bustling city street. But to say that nature is inherently humming with music is to ignore the very real contribution of our own cognitive abilities to the composition of this symphony. ■



Bob Grant
Senior Editor
Special Issue Coordinator

ANDRZEJ KRAUZE

Speaking of Science

Without music, life would be a mistake.

—Friedrich Nietzsche, *Twilight of the Idols* (1889)

Anatomists today would be hard put to identify the brain of a visual artist, a writer or a mathematician—but they would recognize the brain of a professional musician without a moment's hesitation.

—Oliver Sacks, *Musicophilia: Tales of Music and the Brain* (2007)

We're all synesthetes—except we don't know it. Cross-connection is the rule for all brains. Synesthetes just have more of it.

—Richard Cytowic, George Washington University neurologist and one of the leading researchers on synesthesia (Smithsonian.com, January 5)

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Science is more than a body of knowledge; it is a way of thinking. I have a foreboding of an America in my children's or grandchildren's time—when the United States is a service and information economy; when nearly all the key manufacturing industries have slipped away to other countries; when awesome technological powers are in the hands of a very few, and no one representing the public interest can even grasp the issues; when the people have lost the ability to set their own agendas or knowledgeably question those in authority; when, clutching our crystals and nervously consulting our horoscopes, our critical faculties in decline, unable to distinguish between what feels good and what's true, we slide, almost without noticing, back into superstition and darkness.

—Carl Sagan, *Demon-Haunted World: Science as a Candle in the Dark* (1995)

Science is not a political construct or a belief system. Scientific progress depends on openness, transparency, and the free flow of ideas.

—Rush Holt, CEO of the American Association for the Advancement of Science, at a Congressional hearing on the future of the US Environmental Protection Agency (February 8)

[Pardis Sabeti and Houra Merrikh] represent the future of science in this country. They show that by preventing people from Iran from coming to this country, we're hurting our chances to excel in science. And contributions in science translate to economic gains.

—Jan Vilcek, New York University biologist and founder of an organization that raises awareness of immigrant contributions to biomedical research, on the danger of President Donald Trump's ban on immigrants from seven Muslim-majority countries, one of which is Iran (*The Atlantic*, January 29)

Confirming CRISPR

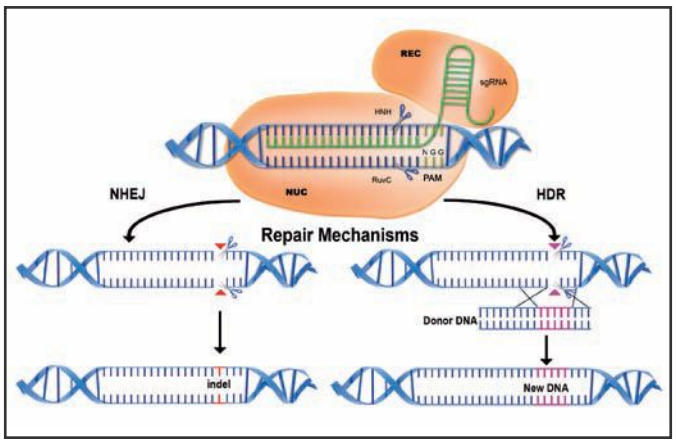
Tools for Efficient and Accurate Experimental Validation

In just a few short years, CRISPR/Cas9 has gone from a prokaryotic antiviral defense mechanism to transforming genome editing. Whether you are considering how to incorporate CRISPR into your workflows or already using it, establishing a robust and reliable validation process for your CRISPR/Cas9-directed gene editing is critical. You need to be able to trust that any observed effects in your newly generated experimental model can be accurately attributed to the desired genome manipulation. Due to technological advancements, several popular CRISPR-validation methods are available today, including digital-droplet polymerase chain reaction (ddPCR), high resolution melt (HRM) PCR, and capillary electrophoresis (CE).

PCR-BASED OPTIONS

Before you commit to a technique for CRISPR validation, consider how often you will be conducting these analyses. Large-scale and small-scale users have differing needs and considerations, and will select techniques and instruments accordingly. While large-scale users are more concerned with efficiency and throughput, small-scale users are looking for a streamlined, intuitive, and cost-efficient apparatus. Sensitivity and selectivity are key as well, and precision requirements must be carefully weighed against throughput needs and costs.

ddPCR provides very low detection thresholds and generates highly precise results by bypassing serial-dilution errors, amplification-efficiency inconsistencies, and increasing the number of data points per run. However, its multi-instrument workflow necessitates increased human input and limits throughput capacity. Conversely, while HRM is intuitive and streamlined, it has a high detection threshold and is susceptible to analysis ambiguity if the amplicon melt curves are not clustered.



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In addition to high sensitivity and specificity, the Fragment Analyzer provides variable throughput capacity, fitting any throughput demand. For example, the Fragment Analyzer can store and automatically run multiple plates sequentially or run a single row of a 96 well plate, greatly increasing laboratory efficiency. Both HRM and the Fragment Analyzer enable real-time visualization of assay results.

Cost efficiency and instrument versatility are greater concerns for smaller-scale users, and CE technology can be adapted to accommodate this need. One option recently made available are the optimized AccuCleave™ family of kits from Advanced Analytical, which use a validated heteroduplex-cleavage assay with options for detection on the Fragment Analyzer or standard gel electrophoresis.

SET YOURSELF UP FOR SUCCESS

Whether you are looking to start using CRISPR in the lab, improve experimental accuracy, or increase sample throughput, it is important to identify your own requirements and select what best fits your needs. This should not be a lonely process: talk to equipment manufacturers regarding their capabilities, and work with them to establish the perfect fit for you. Finally, keep an eye out for adaptability and flexibility. Science does not stand still, and neither does your laboratory. Plan ahead for what the future may bring, and identify what can move with you.

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Notebook

ALAN SCHULTZ, BAYLOR UNIVERSITY

SWEET SOUNDS?: Ricardo Godoy tests the auditory preferences of a Tsimane' man in Bolivia.

Do You Hear What I Hear?

Deep in the Amazon rainforests of Bolivia live the Tsimane', a tribe that has remained relatively untouched by Western civilization. Tsimane' people possess a unique characteristic: they do not cringe at musical tones that sound discordant to Western ears. The vast majority of Westerners prefer consonant chords to dissonant ones, based on the intervals between the musical notes that compose the chords. One particularly notable example of this is the Devil's Interval, or flatted fifth, which received its name in the Middle Ages because the sound it produced was deemed so unpleasant that people associated it with sinister forces. The flatted fifth later became a staple of numerous jazz, blues, and rock-and-roll songs.

Over the years, scientists have gathered compelling evidence to suggest that an aversion to dissonance is innate. In 1996, in a letter to *Nature*, Harvard psychologists, Marcel Zentner and Jerome Kagan, reported on a study suggesting that four-month-old infants preferred consonant intervals to dissonant ones. Researchers subsequently replicated these results: one lab discovered the same effect in two-month-olds and another in two-day-old infants of both deaf and hearing parents. Some scientists even found these preferences in certain animals, such as young chimpanzees and baby chickens.

"Of course the ambiguity is [that] even young infants have quite a bit of exposure to typical Western music," says Josh McDermott, a researcher who studies auditory cognition at MIT. "So the counter-argument is that they get early exposure, and that shapes their preference."

McDermott and his colleagues decided to investigate whether a preference for consonant tones was truly a hardwired trait by testing musical preferences in the Tsimane', whose limited access to television and radio gives them minimal exposure to Western music.

They asked 64 Tsimane' villagers how pleasant they found a series of recorded and synthetic tones, and found a sur-

prising result: unlike participants in the U.S., in a nearby rural town, and in La Paz, the capital of Bolivia, they could hear the difference but did not show any preference for consonance over dissonance. Even when the team repeated the experiment with a separate group of 50 villagers using modified versions of their own tribal songs, they found the same results (*Nature*, 535:547-50, 2016).

We know that there are different contributing factors, and we know that musical exposure is important, but we don't understand exactly how.

—Nori Jacoby, Columbia University

During multiple recording sessions with the Tsimane' musicians, the team learned some of the unique qualities of the tribe's music that could have contributed to their distinctive musical preferences. "One of the things that's really interesting about their music is that . . . unlike most other cultures, they don't do group performances," McDermott says. "That was one of the reasons why they were an interesting test group for this question, because we had reason to think that they would not have experienced harmony before, at least not in a significant way."

But not everyone is convinced by McDermott and his colleagues. "I don't think it proves that preferences for consonance are not biologically driven—I think that it probably has a strong biological component, but that doesn't mean experience can't overcome it," says Laurel Trainor, a neuroscientist who studies auditory development at McMaster University in Ontario, Canada. Trainor adds that even in Western cultures, people differ in how much dissonance they like—jazz musicians, for example, are more likely to enjoy dissonant chords because they play them so often.

"I think at the moment the evidence is still in favor of a consonance preference being potentially innate," says Zentner, who is now at the University of Innsbruck in Austria. "But if this [study] were to be replicated with different stimuli and in other similar remote cultures, it would clearly call this biological theory into question." But regardless of what future work reveals, he says, the group is doing important work, as cross-cultural studies—especially in remote groups unexposed to Western music—are rare.

McDermott has gone back to the Amazon with Nori Jacoby of Columbia University to see whether there are cultural differences in how we perceive rhythms as well. To reveal the Tsimane' people's internal biases, the pair asked participants to listen to a series of random beats and repeat them until rhythms emerged. When they compared these results to those gathered from US participants, they found that people from both cultures shared preferences for the simplest rhythm intervals, such as 1:1:2, where the third beat is twice the length of the first two (one clear example of this is the opening sequence of "Jingle Bells").

However, some biases intrinsic to Americans were not found in the Tsimane', and vice versa. These differences corresponded to the characteristics of the cultures' music. For example, the 3:3:2 ratio, which, according to Jacoby, is commonly found in Western pop music, was uniquely preferable to listeners from the U.S. (*Curr Biol*, doi:10.1016/j.cub.2016.12.031, 2017).

"We know that there are different contributing factors, and we know that musical exposure is important, but we don't understand exactly how," says Jacoby. "One of the things that we are really excited about is to repeat the same experiment in multiple cultures." For now, the researchers plan to return to Bolivia to conduct further investigations with the Tsimane'. A question Jacoby says he hopes to answer on his next trip is whether the tribe's rhythmic biases are due to the types of song that the Tsimane' sing.

McDermott is interested in finding out whether the Tsimane's preferences change over time, especially as more Western music gets introduced into their villages. "There's quite a bit of modernization, [and] the Bolivian government is trying to wire

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*BioSafe and Biosafety are terms intended to describe the enhanced biocontainment features of our products.

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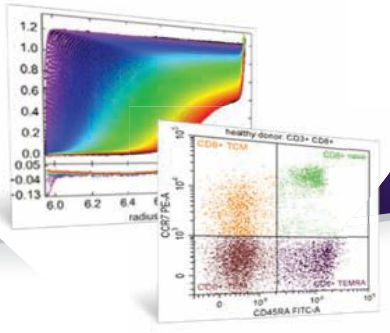
CENT-2190PST12.16

Jay, however, wanted to **characterize his options.**



Data provided by the Optima AUC (left) and CytoFLEX Flow Cytometer (right).

Jay was passionate about thoroughly getting to know all types of particles—from nanovesicles to macromolecules, and countless others in between.

That's why the Optima AUC analytical ultracentrifuge caught his eye. He quickly knew they were on the same wavelength (actually 20 wavelengths), and he'd never gotten information faster.

But another characterization technique—the CytoFLEX Flow Cytometer**—presented optimal excitation too. Never before had Jay seen a research platform that pushed the boundaries of what's possible with benchtop flow cytometry.

Jay wanted to see as much as possible.

Meet your centrifuge match

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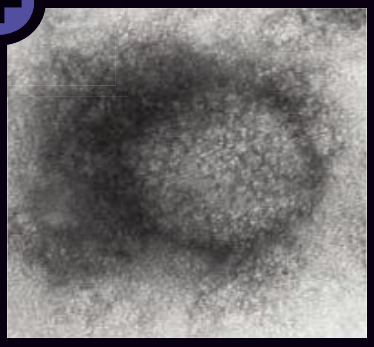
CENT-2190PST12.16

Going Viral



- **FAVORITE SONG:** "Express Yourself" – Madonna
- **SAFETY:** BSL-2
- **STABILITY:** Transient
- **CELL-TYPE PREFERENCE:** Free spirit (open to relationships with all cell types)
- **GENOME SIZE:** 36 – 39 kb
- **INSERT CAPACITY:** 8 kb
- **RELATIONSHIP STATUS:** I won't integrate into your DNA. Don't integrate into mine!

PREFERRED VIRUS #1

ADENOVIRUS

I like big transgenes and I cannot lie.

FRIENDS: 2

GENE + ADD FRIEND

2 HELPER-DEPENDENT ADENOVIRUS + ADD FRIEND

MESSAGES:

HELPER-DEPENDENT ADENOVIRUS

What's wrong, HDA?

Someone called me gutless today. I don't know why! I'm pretty brave.

Aw, they were just talking about your ability to carry up to 32 kb of foreign DNA!

Enter your message here, please SEND

O.K. Capsid: Where Busy Scie

Choosing a viral delivery system is a bit like choosing a partner. Do yo
carry a bit of your genetic material? But like any relationship, you nee

- **FAVORITE SONG:**
  "Jack of All Trades"
  – Bruce Springsteen
- **SAFETY:**
  BSL-2+
- **STABILITY:**
  Transient or stable
- **CELL-TYPE PREFERENCE:**
  I'm pretty picky with my type, but if you're witty, caring, and enjoy crowds, we're probably well suited (Brain, Helper T cells, Intestinal epithelia, Macrophages)
- **GENOME SIZE:**
  9.2 kb
- **INSERT CAPACITY:**
  10 – 11 kb
- **RELATIONSHIP STATUS:**
  Love me, love my packaging plasmid

PREFERRED VIRUS #2

LENTIVIRUS

I'm a V.I.P. – a very important particle, so I get through the door at all of the best spots.

FRIENDS: 4

GENE + ADD FRIEND	FOAMY VIRUS + ADD FRIEND
RETROVIRUS + ADD FRIEND	POXVIRUS + ADD FRIEND

MESSAGES:

FOAMY VIRUS

Hey Len, what's your secret to such highly efficient delivery to neurons?

It's simple, really. I just slip in at the nuclear pore!

Enter your message here, please

SEND

ntists go to Meet the Virus of T

ou share goals? Are you truly compatible? Have you seriously considere
ed to do your homework before inviting a viral delivery system back to y

- **FAVORITE SONG:** "Safety Dance" – Men Without Hats
- **SAFETY:** BSL-1 (without helper virus)
- **STABILITY:** Transient
- **CELL-TYPE PREFERENCE:** Open minded (open to relationships with all cell types)
- **GENOME SIZE:** 4.7 kb
- **INSERT CAPACITY:** 4.5 kb
- **RELATIONSHIP STATUS:** Perpetual Third Wheel

PREFERRED VIRUS #3

ADENO-ASSOCIATED VIRUS

Safety first! Packaging second... or third.

FRIENDS: 4

GENE + ADD FRIEND	ADENOVIRUS + ADD FRIEND
HERPES SIMPLEX VIRUS + ADD FRIEND	EPSTEIN-BARR VIRUS + ADD FRIEND

MESSAGES:

EPSTEIN-BARR VIRUS

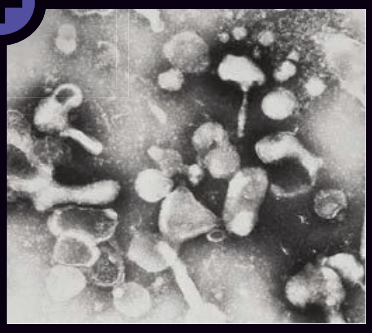
I'm so tired of all this talk about the hazards of viral gene delivery.

Me too! That's why I'm starting a campaign to limit genome integrations to chromosome 19 where they have no discernable effect!

Enter your message here, please SEND

neir Dreams

d asking them to
our incubator.



- **FAVORITE SONG:** "Divide and Conquer" – Hüsker Dü
- **SAFETY:** BSL-2+
- **STABILITY:** Stable
- **CELL-TYPE PREFERENCE:** Looking for an active partner... actively dividing, that is
- **GENOME SIZE:** 9 kb
- **INSERT CAPACITY:** 8 kb
- **RELATIONSHIP STATUS:** Serial Monogamy

PREFERRED VIRUS #4

RETROVIRUS

Division is a decision, and I choose dividing cells!

FRIENDS: 4

- GENE — + ADD FRIEND
- LENTIVIRUS — + ADD FRIEND
- FOAMY VIRUS — + ADD FRIEND
- REVERSE TRANSCRIPTASE — + ADD FRIEND

MESSAGES:

LENTIVIRUS

You're such a show off! Reverse transcription is all smoke and mirrors.

Oh yeah?
Why don't you say hello to my little friend? @RT

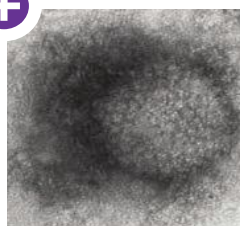
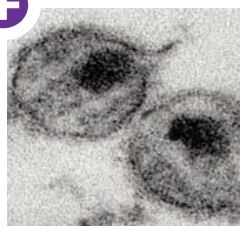
REVERSE TRANSCRIPTASE

Hello

Enter your message here, please SEND

Going Viral

Where Busy Scientists go to Meet the Virus of Their Dreams

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up their villages with electricity," McDermott says. "I think there will be potentially big changes that will happen, which could be interesting to track." —Diana Kwon

Hearing Things

A few years ago, UK composer and technology reporter LJ Rich participated in a music technology competition as part of a project with the BBC. The 24-hour event brought together various musicians, and entailed staying awake into the wee hours trying to solve technical problems related to music. Late into the night, during a break from work, Rich thought of a way to keep people's spirits up.

"At about four in the morning, I remember playing different tastes to people on a piano in the room we were working in," she says. For instance, "to great amusement, during breakfast I played people the taste of eggs."

It didn't take long before Rich learned, for the first time, that food's association with music was not as universally appreciated as she had assumed. "You realize everybody else doesn't perceive the world that way," she says. "For me, it was quite a surprise to find that people didn't realize that certain foods had different keys."

Rich had long known she had absolute pitch—the ability to identify a musical note, such as B flat, without any reference. But that night, she learned she also has what's known as synesthesia, a little-understood mode of perception that links senses such as taste and hearing in unusual ways, and is thought to be present in around 4 percent of the general population.

It's a difficult phenomenon to get to the bottom of. Like Rich, many synesthetes are unaware their perception is atypical; what's more, detecting synesthesia usually relies on self-reported experiences—an obstacle for standardized testing. But a growing body of evidence suggests that Rich is far from being alone in possessing both absolute pitch and synesthesia. Some researchers are beginning to explore potential biological links between the traits, not only to gain insight into synesthesia itself, but as a window into how the brain develops a perception of the world in the first place.

"The two conditions seem superficially quite different," says Psyche Loui, a psychologist and neuroscientist at Wesleyan University, adding that most synesthesia researchers focus on "grapheme-color" associations—the experience of seeing colors when reading words or numbers. "That's a visual-to-visual association," she says. "Absolute pitch is auditory. But when you think about them in general terms of perception, I think the fact that they might be related becomes more obvious."

A few years ago, Loui, then at Harvard Medical School, and colleagues performed functional magnetic resonance imaging (fMRI) on 30 people with either absolute pitch or tone-color synesthesia—the association of specific sounds with colors—alongside 30 control participants. They found that, while listening to music, people with absolute pitch or synesthesia showed unusual neural activity, particularly in the superior temporal gyrus—a region involved in processing auditory information soon after it arrives at the brain (*ICMPC*, 618-23, 2012).

"There was more activity both in people with absolute pitch and in synesthetes compared to controls," Loui says. And intriguingly, people with "absolute pitch had more in the left hemisphere and [those with] synesthesia had more in the right hemisphere. It led us to think that absolute pitch and synesthesia are really two sides of the same coin."

Her findings fit into a growing appreciation of neural connectivity's role in a number of unusual modes of social and sensory perception, from autism to heightened musical sensitivity. "I suspect [absolute pitch] is one of many types of hyperconnectivity," Loui says. "Synesthesia is another one, exceptional creativity may be a third. They could all be different manifestations of the same brain phenomenon."

It's a view shared by Elena Kowalsky and Peter Gregersen, researchers at the Feinstein Institute of Medical Research in Manhasset, New York, who are tackling the question of synesthesia's biological basis from a genetic perspective. In 2013, their group found that, among 768 people with absolute pitch, around 20 percent also reported experiencing some form of synesthesia—usually the association of colors with particular notes or sounds.

A subsequent linkage analysis revealed that regions on at least two chromosomes

ANDRZEJ KRAUZE

showed associations with both traits, "strongly suggesting [the traits] have common genetics," says Gregersen. "We think [synesthesia] is basically another manifestation of the same type of underlying brain connectivity, with a similar genetic basis" (*Hum Mol Genet*, 22:2097-104, 2013).

The Feinstein researchers are now resequencing regions common to both traits to identify the culpable genes. And they've highlighted some potential candidates, including *EPHA7,* a gene thought to influence neural connectivity in the developing brain.

The scientists are also collecting data from a specially designed cell phone app, PitchMatch!, which assesses users' pitch-recognizing ability and uploads results online. Ideally, the tool will allow for a better estimate of the occurrence of heightened auditory perception in the general population, Gregersen says. And it could one day allow researchers to delve into more complex, unanswered questions about how synesthesia and absolute pitch arise: how genetic predisposition interacts with the environment, for example, and to what extent either of these traits can be learned or encouraged during development.

In the meantime, though, the study of traits such as absolute pitch and synesthesia continues to expand science's understanding of how the brain builds a perception of reality. "I think we take it for granted that we all perceive the world in the same way," notes Kowalsky. Exploring the range of humans' experience of tastes, sights, and sounds "opens up this whole window into 'What is perception?'" she says. "What does it mean to see the world through this different lens?"

—Catherine Offord

Furry Fans

After decades of studying primate behavior at the University of Wisconsin–Madison, Charles Snowdon closed his colony of cotton-top tamarins (*Saguinus oedipus*) in 2008. The little monkeys, looking like better-groomed versions of Spike from the movie *Gremlins*, had given Snowdon a glimpse into various aspects of their social lives, from parenting and social learning to hormones and vocal communication. But one of his last studies on the species took him in a totally different direction.

Several years earlier, Snowdon had received a call completely out of the blue from a cellist with a scientific bent named David Teie, who had been studying the various components of music and how each relates to the human experience and affects our emotions. Take pulse, for example—the maternal heartbeat to which every fetus develops. "Even though it's not part of our language, you can find it in all [forms of] music," says Teie.

He explains that there are about two dozen of these musical components, acting like ingredients for song recipes, and Teie felt he had reasonable explanations for why each of them is part of human music. "If I had indeed figured out the recipe [for humans], I should be able to replace the ingredients with ingredients designed for another species," Teie reasoned. And so he called up Snowdon to ask for help.

The cellist asked the biologist if he would be willing to test the effects of music on the monkeys. Snowdon liked the idea. The tamarins in his colony had never been exposed to human music. "They were completely naive and, therefore, they would be good test subjects to test emotionality," Snowdon says. Teie composed several songs, two mellow and two upbeat, using what he imagined to be tamarin musical ingredients, such as tempos matching their calls and tones that exist in their vocal range.

When Snowdon played snippets of the songs for the animals, he found the monkeys displayed an increase in anxious behaviors after the energetic songs. And compared with baseline behavior, they appeared more relaxed after the calming music (*Biol Lett*, 6:30-32, 2010). "It suggests animals other than humans can appreciate music, at least the emotional aspects," says Snowdon. "It's a way of arguing that the emotional aspects of music have a long evolutionary history."

Their next project was to test this idea on cats. Teie made music this time with components resembling purrs, suckling sounds, and female vocalizations. "It took five people and four software programs about two weeks to get the two-second sample of the purr I was

DOE, RAY, MONKEY: Charles Snowdon's cotton-top tamarins showed emotional responses to music written expressly for them.

MEOWTZART: David Teie recorded music that incorporated feline vocal samples in his bathroom/recording studio.

really happy with, that had a pitch in it and all the contours I wanted," says Teie. His hard work paid off; when cats were presented with various songs, they gravitated toward a speaker playing music Teie wrote for the cats more than toward a speaker playing Bach's *Air on a G String* or Gabriel Fauré's *Élégie* (*Appl Anim Beh Sci*, 166:106-11, 2015).

"Since animals do not typically perceive sounds in the same way as people, it makes a lot of sense to design music that is more tailor-made for the species under target," Deborah Wells, who studies animal behavior at Queen's University Belfast, wrote in an email to *The Scientist*. In her own work, she's found that dogs in kennels and elephants and gorillas kept in zoos seem to benefit from classical music. But she also says that other studies have found no effect, or that certain types of music can agitate animals. "It is still unclear how music exerts its effects on animals, and more research is needed to explore the potential mechanism/s by which acoustic stimuli influence animal well-being."

Emma Wallace, a graduate student at the University of York, says there's been evidence that music has a positive effect on chimpanzees' welfare. One possibility is that music might mask unpleasant sounds, say, of a ventilation system or a noisy animal shelter. In a recent study, she wanted to see how zoo-housed chimpanzees would react. She had pop and classical music played in the chimps' enclosures, but the animals didn't show much of a response one way or another. "Generally, it looks like music is not something that they're enjoying," she says, "but it's not having a negative effect on their welfare either."

Roian Egnor, who studies mouse vocalization at Janelia Research Campus, is skeptical about nonhuman animals' enjoyment of music. "Any sound at all is going to interfere with your ability to hear a predator coming," she says. "My bet? I would need extraordinary evidence to show that an animal actually likes music. I'd love to see it."

Teie, for one, was convinced by Snowdon's behavioral data that the feline test subjects were indeed drawn to his music. So his next step, obviously, was to make them an album. *Music for Cats* dropped in October of last year, earning a spot on the UK's Top 40. It was the first time music intended for animals made the chart, he claims, "so in that sense it's the most popular animal music ever," he says jokingly. "But Adele is not worried about the competition."

—Kerry Grens

Additional reporting by Joshua A. Krisch

Learning to Jam Again

Ray Goldsworthy lost his hearing when he was 12 years old. A case of spinal meningitis caused swelling in his central nervous system that resulted in irreparable damage to the neurons of the cochlea, the inner-ear structure that converts incoming sound waves into neural signals sent to the brain. A year later, Goldsworthy became one of the first children to receive a cochlear implant as part of a 1987 pediatric trial. He could hear again, but his auditory sense wasn't the same.

A lot of things about using a cochlear implant (CI) take getting used to. The devices comprise an external microphone, a sound processor, and an electrode array that stimulates the cochlear nerve according to the output of an algorithm programmed into the processor. Understanding conversation in a noisy background can be challenging, even for experienced CI users. Music sounds strange, and sometimes unappealing, like noise. One common comparison is that it's like listening to someone play the piano with mittens on, or it sounds like you're underwater or in a tunnel, says Goldsworthy, now a researcher at the University of Southern California's Keck School of Medicine: individual instruments are difficult to identify, and lyrics are hard to understand. "Everything's a little bit blurry."

CARLA Y. BOE; COURTESY OF DANIELLA TEIE

IMPLANTING TUNES: Ray Goldsworthy helps patients with cochlear implants regain their ability to enjoy music.

Prior to losing his hearing, Goldsworthy liked to jam on his drum set, and he wasn't about to give up music just because the CI had changed his aural environment. But the rock music he liked involved a lot of different instruments that the CI couldn't translate appropriately for Goldsworthy's brain to understand. "It didn't sound like what I was used to," he says. So, shortly after he got his CI, he started listening to instrumental solos, often of the jazz variety. Then he tried duets. "[I] gradually worked my way back to something as complex as rock and roll or multiple-instrument jazz pieces," he says.

Goldsworthy gives credit to the improvements in technology over the years—mostly changes in the algorithm that translates sound into cochlear stimulation—but, "I think just as important or more important is the experience I've had rediscovering music," he says. "You can relearn to appreciate it, but it has to be an active process."

Cochlear implant listeners think that they perceive the melody worse, that they perceive the instrument detection worse than normal hearing listeners.

—Waldo Nogueira,
Medical University Hannover

His personal experiences motivated Goldsworthy to study cochlear implants as a scientist, and he's always trying to improve the function of his own device. He'll occasionally upload new software to his CI that

he hopes will provide him with new information about what he's hearing. "When I do that, I have to reenter this period of learning," he says. Right now Goldsworthy's working on software that's better at conveying information about the speed of stimulation, rather than simply where along the cochlea the electrodes are firing. "The spatial location of the nerve that's being stimulated is a cue, but also the speed with which you stimulate the nerve is a cue," he says—one that current implants do not use effectively.

Goldsworthy also wants to understand how musical perception—in particular, patients' ability to discern tones—is tied to the ability to understand speech in everyday conversation. In 2015, Goldsworthy compared pitch and phoneme perception in CI users and found the two measures to be positively correlated (*J Assoc Res Otolaryngol*, 16:797-809). "Individuals who [understand speech] better in a noisy situation have better pitch perception," he says.

The exact relationship between music and speech perception remains unclear, however. (See "Musical Roots" on page 26.) Joe Crew of cochlear implant company Advanced Bionics suspects that the correlation may simply reflect general auditory processing abilities, such as focus, fatigue, and working memory. "The link between speech and music is pretty tenuous once you factor that out," he says. Goldsworthy thinks it's worth a try, though. His group is now working to train CI users to better hear music in hopes that it might also improve their ability to understand speech.

Waldo Nogueira of the Medical University Hannover, Germany, thinks this type of music therapy will actually become more common among CI users. "It seems that there is more and more evidence—although it's not accepted completely by the research community—that musicians [or] people having musical training seem to have an advantage in understanding speech in these cocktail party scenarios—in noise," he says. "This suggests that musical training may [help] CI users to improve speech intelligibility in noise." (See "Music Therapy" on page 42.)

But it's not all about improving speech perception. Improving the music-listening experience for CI users is another important goal. And if music is more enjoyable, it will make for a more effective therapy.

One approach to making music more CI-friendly is to remix it—basically, take apart the music's components (often laid down as separate tracks) and recompose them in a simpler arrangement. Wim Buyens of the Cochlear Technology Centre in Belgium and colleagues have found, for example, that raising the volume on vocals and removing harmonic instruments from pop music improves the listening experience for CI users (*IEEE Trans Biomed Eng*, 62:2434-42, 2015).

Meanwhile, Nogueira is also putting on music concerts specifically composed for the enjoyment of CI users. He and his colleagues in the Auditory Prosthetic Group at the university's German Hearing Center organized meetings between composers and CI users, who shared information about the technology and their experiences with music. The musicians then wrote electroacoustic numbers inspired by what they'd learned and by the patients themselves. "We were aiming at music that can be similarly perceived by CI users and normal hearing listeners," says Nogueira.

At each of two concerts held so far (musIC 1.0 in February 2013 and musIC 2.0 in February 2015), the pieces, often paired with visualizations or other performances, were played over loudspeakers to an audience of 250 normal hearing listeners and CI users. In addition to promoting CI awareness and encouraging CI users to listen to music, the concerts "are also experiments," Nogueira says. After each musical number, the audience was asked to fill out a questionnaire that touched on the technical aspects of the music, the participant's subjective impressions of music, and their emotional responses to it. The 90 CI users and 168 normal hearing listeners who responded gave similar scores on the last two counts, "but when it comes to the technical aspects of the music, there was a significant difference," says Nogueira. "Cochlear implant listeners think that they perceive the melody worse, that they perceive the instrument detection worse than normal hearing listeners."

—Jef Akst

The Hidden Costs of New Drugs

What can be done to expedite the development of novel therapeutics and reduce how much consumers must pay for these treatments?

BY JOHN D. LOIKE AND JENNIFER MILLER

Drug approvals by the US Food and Drug Administration (FDA) dropped dramatically in 2016—down by 57 percent over the year before. While some of this decline was due to a record number of approvals in 2015, only 22 novel drugs were approved last year—fewer than in each of the previous five years. Striving to make returns on their investments—to gain FDA approval for a novel therapy averages around $2.6 billion and 10 years—pharmaceutical companies sometimes hike drug prices to offset low productivity. Prescription drugs are the fastest growing health-care expense, with costs increasing by 9 percent from 2014 to 2015, according to the latest report from the Centers for Medicare & Medicaid Services (CMS).

In addition to translating to high medication prices for patients, the exorbitant cost of drug development can result in many other unwanted outcomes. First, it can financially strain small biotechs to the point that the companies can't fund their own Phase 2 or 3 clinical trials and are forced to go to pharmaceutical giants for financial help. High costs can also deter companies, big and small, from innovating and researching therapeutics for small or low-income patient populations.

Although the FDA has made efforts to reduce drug development costs and offers incentives for pharma to develop less-profitable drugs, such as those for orphan diseases, many in the industry believe these steps have not made a significant financial impact. As we go to press, President Donald Trump is considering appointing Jim O'Neill, among other candidates, as the new head of the FDA. As managing director of Peter Thiel's Mithril Capital, O'Neill has publicly proposed eliminating the FDA's requirement for Phase 2 and 3 trials, in an effort to lower drug development costs.

Among other things, O'Neill favors "progressive approval" of drugs, similar to the methods employed in Japan, where medicines can be approved following proof of safety. Once a drug is in use, companies in Japan move to a Phase 4, post-market assessment to monitor their patients and regularly disclose efficacy data.

We argue that eliminating Phase 2 and 3 clinical trials would be unwise for a number of reasons. Chief among them, testing drugs in fewer than 100 healthy volunteers—as is done in a typical Phase 1 trial—is not sufficient for identifying health risks in a target patient population. Moreover, Phase 2 trials, for conditions other than oncology, are not that expensive because they involve a limited number of patients. The costs of Phase 3 randomized control trials, in contrast, are high, in part because

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they require the recruitment of hundreds or thousands of patients, often across multiple states and countries, and require a lot of time in assessing a drug's efficacy.

The FDA could explore using adaptive licensing, whereby a drug is provisionally approved for a defined short period of time based on Phase 1 and 2 data. During the provisional approval period, efficacy data are collected directly from clinical use, accruing real-world information capable of overcoming the generalizability limitations of traditional trials. After a year or two, the drug is either fully approved or loses its marketing license.

In lieu of eliminating late-stage trials, there may be other steps that the government can take to address the current regulatory challenges. First, pragmatic and adaptive trial designs could help reduce the numbers of research subjects needed to test a drug and increase the likelihood that trial data are generalizable to the patient population that will use a drug following FDA approval. Currently, many trials are conducted on highly specialized patient populations—for example, healthier and younger than typical patients—causing concerns about the

quality of our medical evidence at the time a new drug is introduced to market.

Second, the FDA could explore reducing the use of animal models in preclinical research. Currently, the FDA requires extensive and sometimes expensive animal trials before entering human studies. While such experiments often detect unwanted side effects, many animal models of diseases are

Clinical trials can cost up to $4 billion dollars and take 10 to 15 years. Fewer than 10 percent make it to market.

poorly translated into successful human trials. As human organoid technology continues to be developed and improved, these human mini organs may prove to be a better and cheaper model system to test the effects of drugs instead of using expensive and often inaccurate animal models.

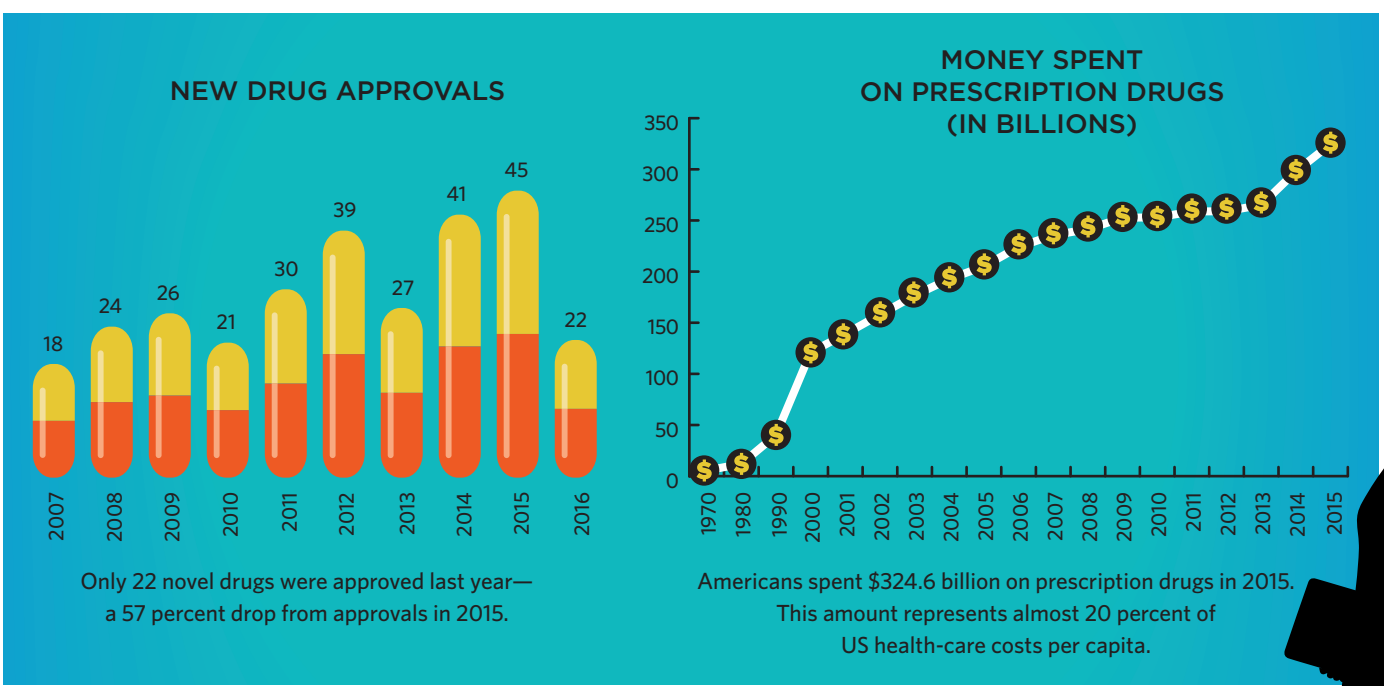
Third, academic institutions and pharmaceutical companies should be required to publish the results of all clinical trials within one year of a trial's primary completion date and to make their patient-level data available to all researchers and physicians. Currently, only 57 percent of trials for new drugs are registered and only 65 percent have publicly disclosed results. Fostering more open science is likely to save money and spur innovation, as researchers learn from the lessons of others and avoid duplicating costly trials.

Fourth, greater adoption and integration of electronic health records with research protocols could help identify new uses for existing drugs, by enabling researchers to collate data on off-label use, for example, and expedite approvals for these indications. The savings that come with repurposing existing drugs can be dramatic: thalidomide, originally approved in Europe in the 1950s as a sedative and in the U.S. in 1998 to treat leprosy, gained FDA approval for the treatment of multiple myeloma in 2006 for less than $80 million.

Last, Congress could explore creating or partnering with an offshoot-pricing agency for new drugs, separate from the FDA and similar to the U.K.'s National Institute for Health and Care Excellence. This agency could monitor drug prices and help assess their overall value, comparative effectiveness, and cost-effectiveness. The Institute for Clinical and Economic Review may be a potential partner. The organization is creating value-based price benchmarks based on the benefits a new drug brings to patients. Last year, PhRMA, the US pharmaceutical trade association, developed principles to guide value assessment frameworks.

There is no doubt that, in general, the FDA has been successful in providing a reliable method for companies to bring new drugs to market. As expensive biotechnology begins to introduce new drug therapies, the agency should consider innovative ways to lower the costs without jeopardizing the safety of patients. ■

John D. Loike is a faculty member at Columbia University College of Physicians and Surgeons. Jennifer Miller is a faculty member at New York University School of Medicine and president of Bioethics International. A version of this story was published at the-scientist.com February 1, 2017.



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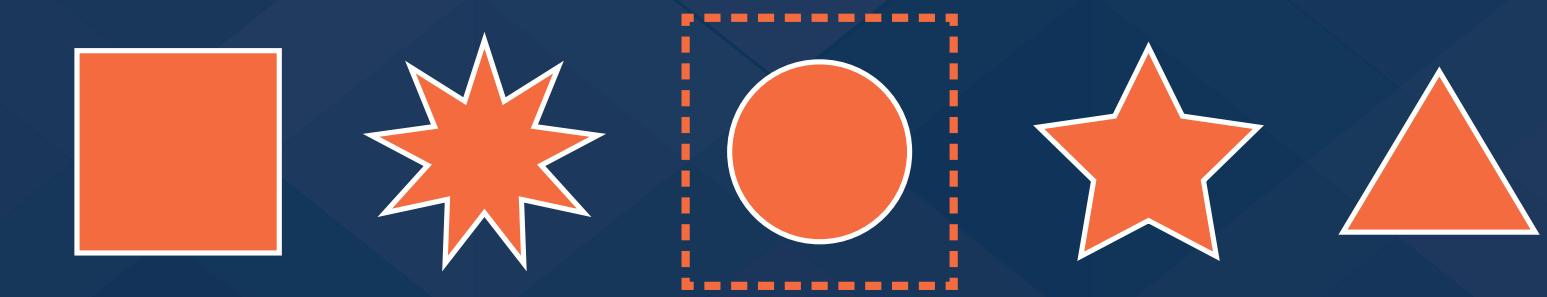

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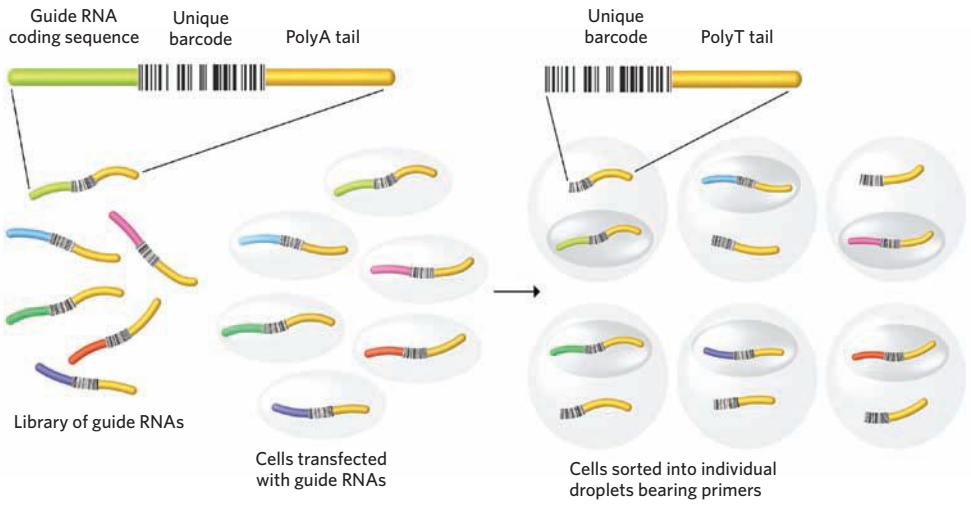
Massively Parallel Perturbations

Scientists combine CRISPR gene editing with single-cell sequencing for genotype-phenotype screens.

BY RUTH WILLIAMS



SINGLE-CELL SCREEN: A library of guide RNAs—each targeting a unique gene for CRISPR-based interference and carrying a unique barcode sequence—is introduced into a population of cells at a concentration that results in one guide RNA entering one cell, on average. Individual cells are then sorted into droplets bearing uniquely barcoded polyT primers, which are used to extract the cell's mRNA. Sequencing the RNA then reveals both the introduced genetic mutations—determined by the guide RNA—and the transcriptional effect of that perturbation—determined by the collection of mRNAs bearing the cell-specific barcode (from the polyT primer).

Determining how the genes in a cell affect its function is the overarching objective of molecular genetic studies. But most genotype-phenotype screens are limited by the number of genetic perturbations that can be feasibly measured in one experiment. In short, the more genetic disruptions examined, the more costly and time-consuming the experiments become.

Indeed, says Trey Ideker of the University of California, San Diego, very few large-scale genotype-phenotype screens have been performed, and those that have were mammoth undertakings. Now, thanks to two highly similar techniques—one called Perturb-Seq, developed by Aviv Regev of the Broad Institute and colleagues, and another, designed by Ido Amit of the Weizmann Institute in Israel and colleagues, called CRISP-Seq—it is possible to study numerous genetic manipulations, individually or combined, in thousands of single cells all in one experiment.

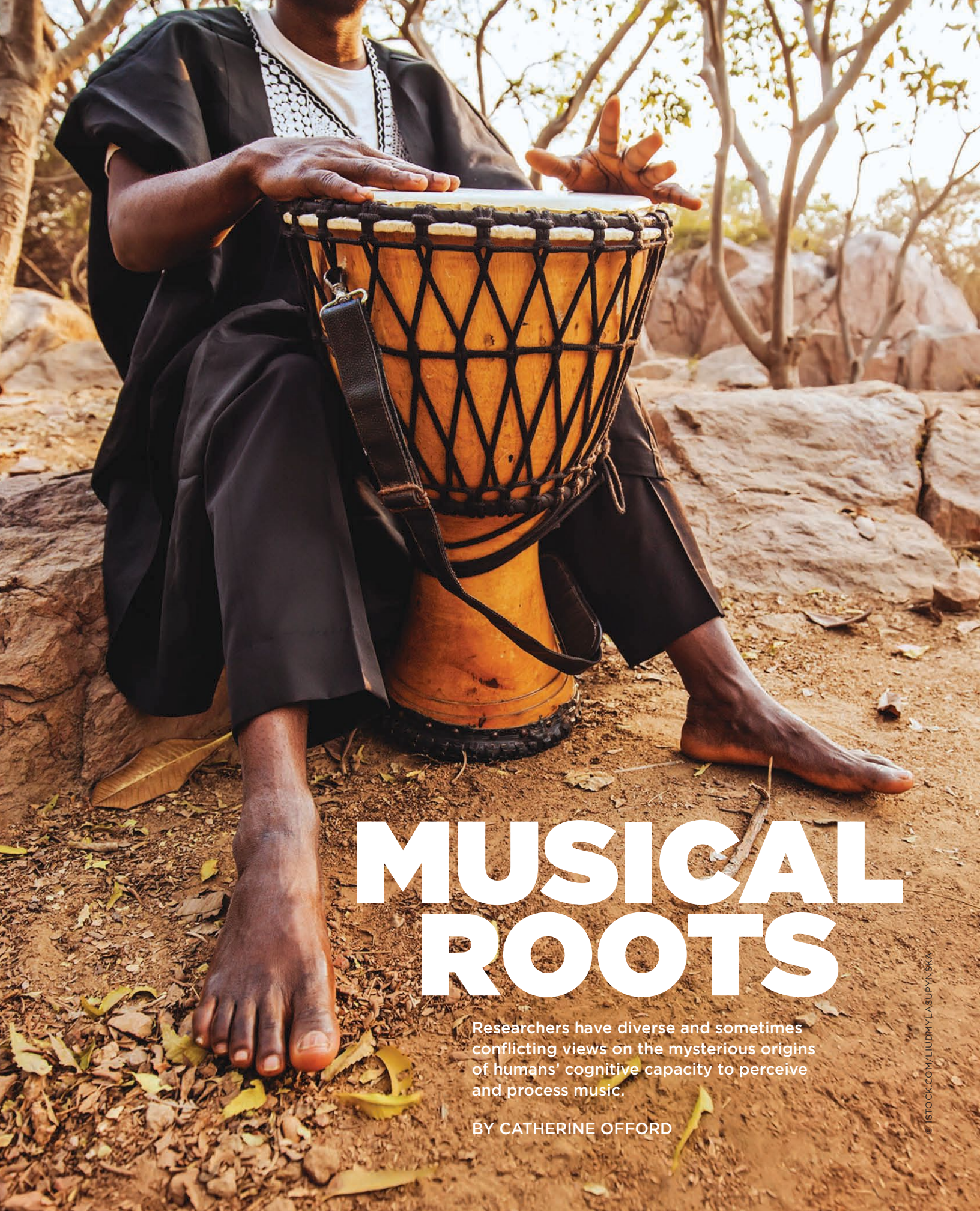
The principle behind Perturb-Seq and CRISP-Seq is to barcode both the individual genetic disturbances and the cells affected, such that sequencing can identify both. Briefly, a library of uniquely barcoded CRISPR guide RNAs targeting genes of interest is introduced into a population of cells. The mRNAs of individual cells are then extracted with uniquely barcoded primers. RNA sequencing reveals both the CRISPR-targeted gene (or genes) and the resulting transcriptional profile of the single cells. Importantly, tens of thousands of these cells can be sequenced in parallel.

Regev and Amit have used their techniques to examine, among other things, transcription factor functions and differentiation regulation in immune cells. But, says Ideker, the possibilities are endless. These are "the first models of this technology," he says, "and they're going to get better and better." (*Cell*, 167:1853-66, 2016; *Cell*, 167:1867-82, 2016; *Cell*, 167:1883-96, 2016) ■

AT A GLANCE

METHOD	GENE DISRUPTION	TRANSCRIPTOME ANALYSIS	ESTIMATED COST*	ESTIMATED TIME*
Gene knockout followed by transcriptome analysis	Many options, including gene editing (CRISPR), random mutagenesis, and homologous recombination	Cells containing disrupted genes are pooled and their mRNAs are extracted and sequenced.	$135,000 to analyze 1,000 gene perturbations by CRISPR	30 hours to analyze 1,000 gene perturbations by CRISPR
Perturb-Seq or CRISP-Seq	CRISPR/Cas9-driven gene editing	Microfluidic technology isolates single cells carrying barcoded gene edits. The cells' mRNAs are then extracted and sequenced with barcoded primers.	$40,000 to analyze 1,000 gene perturbations	12 hours to analyze 1,000 gene perturbations

*Estimations based on unpublished data from Aviv Regev and colleagues



MUSICAL ROOTS

Researchers have diverse and sometimes conflicting views on the mysterious origins of humans' cognitive capacity to perceive and process music.

BY CATHERINE OFFORD

If these properties are absent in some cultures, they can't be strictly determined by something in the biology.

—Josh McDermott, MIT, who collaborated with Ricardo Godoy (pictured at left) to study the musical preferences of the Tsimane' tribe in Santa María, Bolivia

JOSH MCDERMOTT

Getting to Santa María, Bolivia, is no easy feat. Home to a farming and foraging society, the village is located deep in the Amazon rainforest and is accessible only by river. The area lacks electricity and running water, and the Tsimane' people who live there make contact with the outside world only occasionally, during trips to neighboring towns. But for auditory researcher Josh McDermott, this remoteness was central to the community's scientific appeal.

In 2015, the MIT scientist loaded a laptop, headphones, and a gasoline generator into a canoe and pushed off from the Amazonian town of San Borja, some 50 kilometers downriver from Santa María. Together with collaborator Ricardo Godoy, an anthropologist at Brandeis University, McDermott planned to carry out experiments to test whether the Tsimane' could discern certain combinations of musical tones, and whether they preferred some over others. The pair wanted to address a long-standing question in music research: Are the features of musical perception seen across cultures innate, or do similarities in preferences observed around the world mirror the spread of Western culture and its (much-better-studied) music?

"Particular musical intervals are used in Western music and in other cultures," McDermott says. "They don't appear to be random—some are used more commonly than others. The question is: What's the explanation for that?"

Ethnomusicologists and composers have tended to favor the idea that these musical tendencies are entirely the product of culture. But in recent years, scientific interest in the evolutionary basis for humans' musicality—our capacity to process and produce music—has been on the rise. With it has come growing enthusiasm for the idea that our preference for consonant intervals—tonal combinations considered pleasant to Western ears, such as a perfect fifth or a major third—over less pleasant-sounding, dissonant ones is hardwired into our biology. As people with minimal exposure to Western influence, the Tsimane' offered a novel opportunity to explore these ideas.

Making use of the basic auditory equipment they'd brought by canoe, McDermott and his colleagues carried out a series of tests to investigate how members of this community responded to various sounds and musical patterns. The team found that although the Tsimane' could distinguish consonance from dissonance, they apparently had no preference for one over the other. McDermott interprets the results as evidence against a strong biological basis for preference.[1] "If these properties are absent in some cultures, they can't be strictly determined by something in the biology—on the assumption that the biology in these people is the same as it is in us," he says.

But the authors' publication of their results proved controversial. While some took the findings to imply that culture, not biology, is responsible for people's musical preferences, others argued that the dichotomy was a false one. Just because there's variation in perception, it doesn't mean there's no biological basis, says Tecumseh Fitch, an evolutionary biologist and cognitive scientist at the University of Vienna. "Almost everything has a biological basis *and* an environmental and cultural dimension," he says. "The idea that those are in conflict with one another, this 'nature versus nurture,' is just one of the most consistently unhelpful ideas in biology."

Identifying the biological and cultural influences on humans' musicality is one of various thorny issues that researchers working on the cognitive science of music are currently tackling. The field has exploded in recent years, and while many answers have yet to materialize, "the questions have been clarified," says Fitch, who was one of more than 20 authors contributing to a special issue of *Philosophical Transactions B* on the subject in 2015. For example, "rather than talking about the evolution of music, we're talking now about the evolution of musicality—a general trait of our species. That avoids a lot of confusion."

Researchers are beginning to break this trait into various components such as pitch processing and beat synchronization (see Glossary on opposite page); addressing the function and evolution of each of these tasks could inform the broader question of where humans' musicality came from. But as illustrated by the discussions following McDermott's recent publication, it's clear just how much remains mysterious about the biological origins of this trait. So for now, the debates continue.

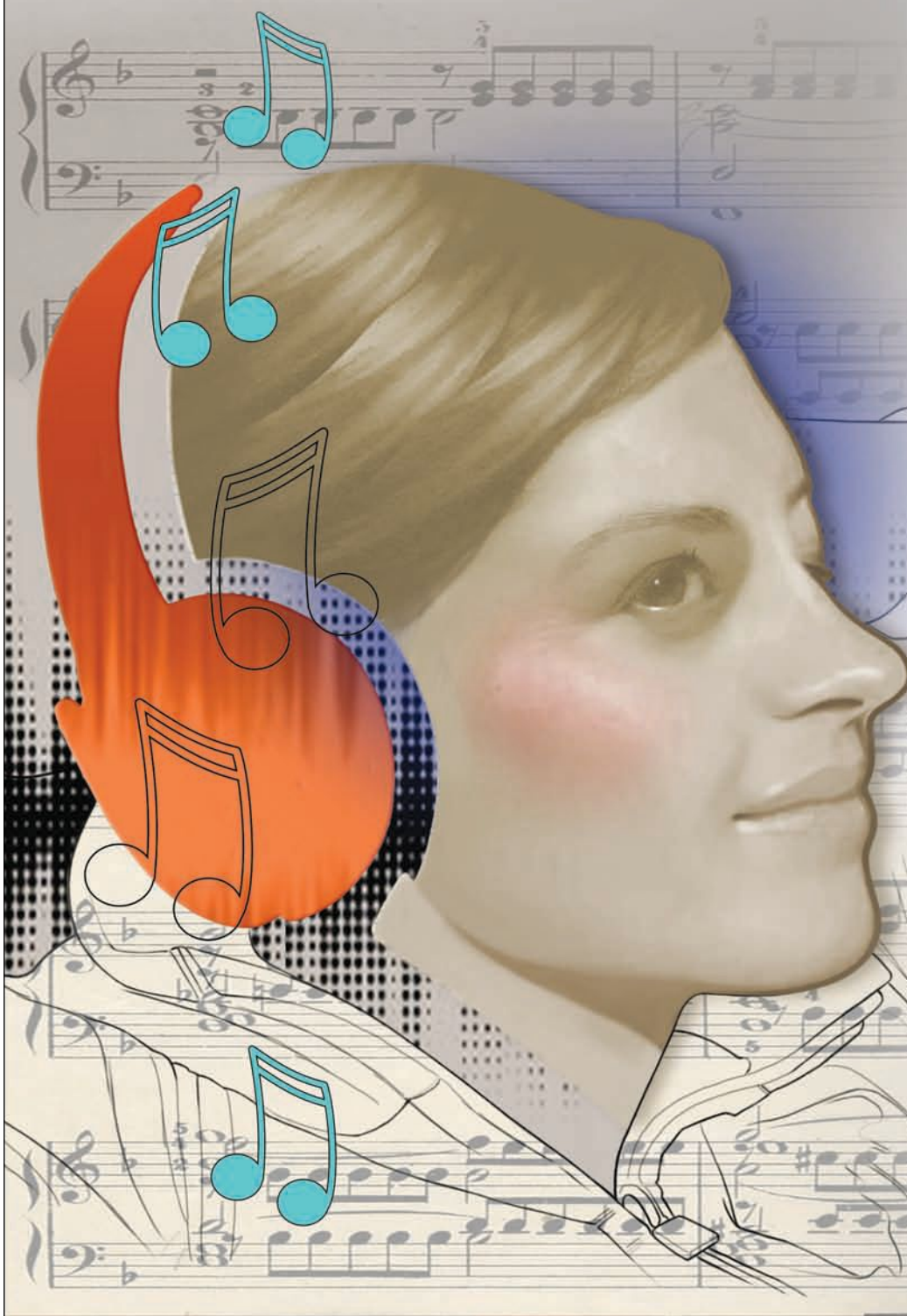
A mind for music?

Musical faculties don't fossilize, so there's little direct evidence of our musical past (see Time Signatures on page 31). But researchers may find clues in the much older study of another complex cognitive trait: speech perception. "Music and language are both sound ordered in time; they both have hierarchical structure; they're in all cultures; and they're very complex human activities," says Fred Lerdahl, a composer and music theorist at Columbia University. "A lot of people, including me, think that music and language have, in some respects, a common origin."

Numerous lines of evidence have supported this view. For example, Tufts University psychologist Ani Patel and colleagues showed a few years ago that patients with congenital amusia, a neurodevelopmental disorder of musical perception commonly known as tone deafness, also had difficulty perceiving intonation in speech.[2] (See "Caterwaul-

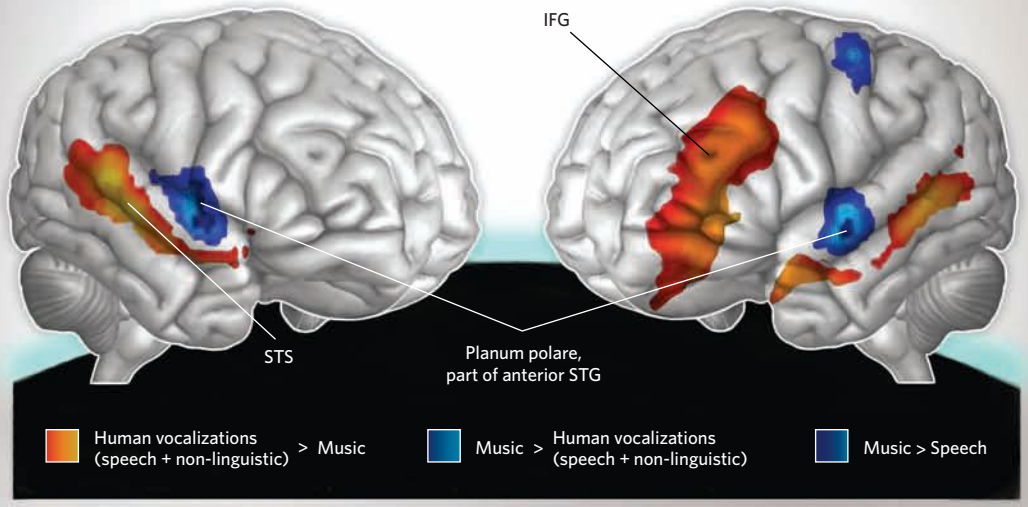
MAPPING MUSICALITY

Huge areas of the brain respond to any sort of auditory stimulus, making it difficult for scientists to nail down regions that are important specifically for music processing. Functional magnetic resonance imaging (fMRI) studies have taken diverse approaches to pinpointing areas involved in musical perception, providing "musical" stimuli ranging from human singing to synthesized piano melodies and other computer-generated sounds, and yielding equally varied results. Despite these hurdles, research is beginning to offer some clues about the regions of the brain involved in musical perception.



MUSIC SPECIFICITY

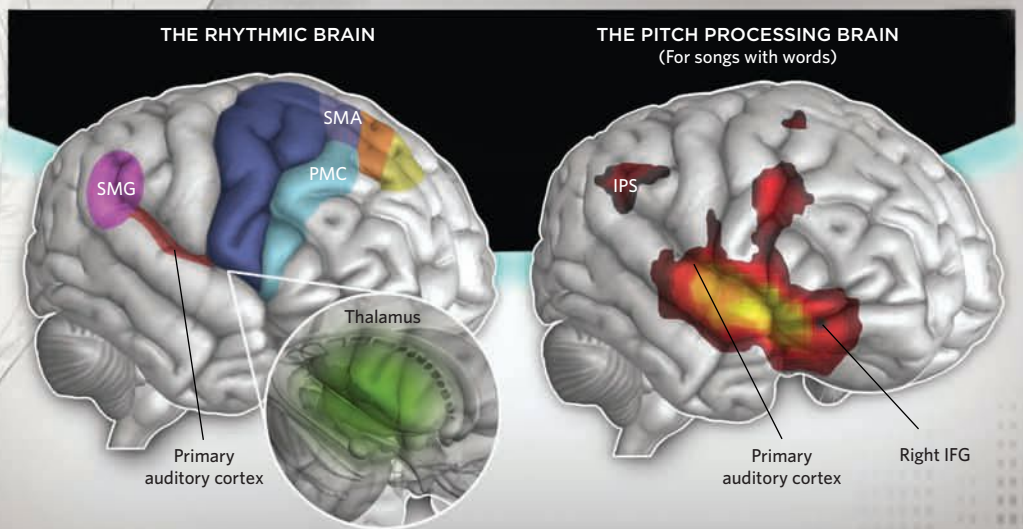
Music activates diverse areas of the brain, from the primary auditory cortex to the amygdala. But the degree to which certain areas are specifically geared to processing music, as opposed to other sounds, is unclear. By comparing activation patterns in the brain while people listened to nonmusical human vocalizations, such as speech or laughter, or to instrumental music, researchers found that certain regions responded more strongly to one type of auditory stimulus than the other. For example, parts of the superior temporal gyrus (STG), the superior temporal sulcus (STS), and the inferior frontal gyrus (IFG) showed stronger responses to vocalizations than to music (orange), while other areas such as the planum polare (part of the anterior STG) showed stronger responses to music than to vocalizations (blue).



BEAT AND PITCH

Some fMRI studies have focused on identifying the brain circuitry underlying specific components of auditory perception. For example, the primary auditory cortex (located in the STG) and the thalamus are thought to play prominent roles in beat perception for both music and speech, and trained musicians may recruit extra language-processing areas such as the supramarginal gyrus (SMG) when listening to complex rhythms. In addition, several regions considered to be part of the motor system have been associated with beat perception, including the supplementary motor area (SMA) and the premotor cortex (PMC), suggesting an important link between perceiving a rhythm and synchronizing movement to it.

Studies of pitch processing, meanwhile, have repeatedly highlighted a role for the auditory cortex, although evidence for the overlap between speech and music in this and other areas is mixed. Some regions, however, including the intraparietal sulcus (IPS, located on the parietal lobe), appear to be activated more by pitch in sung words than by pitch in spoken words. Additional observations revealed differential lateralized activity for song and speech: the left inferior frontal gyrus (IFG), for example, dominates in pitch processing for speech, while the right IFG takes over for song.



GLOSSARY

MUSICALITY
A naturally developing set of biological traits that amount to a capacity to perceive and/or produce music

PITCH PROCESSING
Detection of how high or low a musical note is, either without any reference (absolute pitch) or in relation to the notes surrounding it (relative pitch)

BEAT PERCEPTION
Detection of a regular pulse in music—a prerequisite for synchronizing movements to produce rhythmic drumming or melodies, for example, or to coordinate dance to music

TIMBRE
The sound quality or "color" of a piece of music, achieved by the combination of voices or instruments producing the sounds

TONALITY
A feature contributing to the character of a piece of music, based on the organization of musical tones into scales or keys around one central, tonic note

CONSONANCE
The property of an interval or chord in which musical notes sound in harmony with each other due to their relative frequencies. Consonant intervals are typically considered pleasant, and include perfect fifths, perfect octaves, and major thirds.

AMUSIA
The condition of being unable to recognize or reproduce musical notes. Commonly known as tone-deafness, amusia can be present at birth (congenital) or acquired later in life.

BASED ON *CORTEX*, 59:126-37, 2014. (TOP); *CEREB CORTEX*, 24:836-43, 2014 AND *PHILOS TRANS R SOC LOND B BIOL SCI*, 370:20140093, 2015 (BOTTOM LEFT); *FRONT PSYCHOL*, 3:76, 2012 (BOTTOM RIGHT).

ing for Science" on page 60.) And fMRI scans of normally hearing volunteers listening to recordings have revealed that large areas of the brain's temporal lobes—regions involved in auditory processing—show heightened activation in response to both music and speech, compared with nonvocal sounds or silence.[3] For many, these findings hint at the possibility of common neural circuitry for the processing of speech and music.

But other research points to dissociated processing for at least some components of music and language, suggesting that certain parts of the brain specialized in musicality during our evolution. Lesion studies, for example, show that brain damage can disrupt the processing of pitch in music without disrupting pitch processing in speech.[4] And multivariate neuroimaging analyses with higher sensitivity than traditional methods indicate that, despite stimulating overlapping regions of the cortex, recordings of music and speech activate different neural networks.[5] "People may take localization of activity as evidence for sharing," notes Isabelle Peretz, a neuropsychologist at the University of Montreal. But given the low resolution of most current methods, "that's nonsense, of course."

McDermott's lab recently reported more extreme dissociation. Using a novel approach to analyze fMRI data from people listening to more than 150 recordings of speech, music, nonverbal vocalizations, or nonvocal sounds, the team identified anatomically distinct pathways in the auditory cortex for speech and for music, along with other regions of the brain that responded selectively to each.[6] "We find that they're largely anatomically segregated," McDermott says. "Speech selectivity seems to be located primarily lateral to primary auditory cortex, while music [selectivity] is localized mostly anterior to it."

The neural processing mechanisms themselves remain elusive, but studies like McDermott's "clearly demonstrate that you can separate the representations for speech and music," says Peretz. All the same, she notes, with current research continuing to present evidence both for and against a shared neural basis for music and speech perception, "the debate is still on."

Another way researchers hope to throw more light on how the human brain has become tuned for musical perception is by looking at people's DNA. "For me, [genetics] is the only way to study the evolutionary roots of musicality," says Irma Järvelä, a medical geneticist at the University of Helsinki. In recent years, Järvelä's group has researched genome-wide association patterns in Finnish families. In a preliminary study published last year, the team used standard music-listening tests to characterize participants as having either high or low musical aptitude, and identified at least 46 genomic regions associated with this variation.[7] "We asked, what are the genes in these regions, and are these genes related to auditory perception?" she explains. In addition to homologs of genes associated with song processing and production in songbirds, the researchers identified genes previously linked with language development and hearing.

Music's universality in humans, combined with its fundamental social and cultural roles, is convincing evidence to some that our musicality is adaptive.

Further clues about musicality's genetic basis could come from the study of amusia. In 2007, Peretz and colleagues reported that congenital amusia runs in families.[8] And recent descriptions of high amusia incidence in patients with genetic diseases such as Williams-Beuren syndrome, a condition associated with deletion of up to 28 genes on chromosome 7, may lead researchers to additional musicality-linked genes.[9] "We are making progress along these lines, but there's a lot more to be done," says Peretz. "It's really hard to do, and more expensive than neuroimaging. So we have to be patient." But it's progress worth waiting for, she adds, as an understanding of the genetics contributing to particular musical—or amusical—phenotypes could offer an entirely new perspective on the biological basis for musicality.

Meanwhile, some researchers advocate looking to related species to answer questions about the origins of human musicality. Although nonhuman primates share our ability to distinguish between consonance and dissonance, many apes and monkeys have surprisingly different auditory processing. "Things that are fundamental to music that people thought would be ancient, general aspects of how animals process sound turn out not to be, and potentially reflect specialization in our brains," says Patel. For example, the ability to synchronize movement to a beat, a capacity central to music, "doesn't come naturally to our closest living relatives," says Patel, though he adds that "it does come quite naturally to some other species," including parrots, seals, and elephants. (See "John Iversen: Brain Beats" on page 53.)

Similarly, vocal learning—potentially a requirement for musicality—is known to be prevalent in several taxa, including some species of songbirds, parrots, whales, seals, bats, and elephants, but it is not well documented in any primate other than humans. (See "Singing in the Brain" on page 50.) "It raises the question of why," Patel says. "What basic features of music perception are shared with other species, and what does that tell us about the evolution of those features?"

TIME SIGNATURES

Without physical evidence of ancient humans' musical perception, researchers look for signs of our capacity to produce music to approximate the timescale of musicality's evolution. One way to do this is through archaeology. The oldest undisputed musical instruments are bone flutes (pictured at right) found in caves in Germany that have been dated as more than 40,000 years old (*J Hum Evo,* 62:664-76, 2012). But many researchers argue that the use of the voice as an instrument likely came much earlier than that.

To put an upper limit on the age of vocal musicality, some have turned to human anatomy. Producing complex vocalizations requires both a powerful brain and specialized vocal machinery. During hominin evolution, for example, the thorax become more innervated, a change that allowed humans (and Neanderthals) to more effectively control the pitch and intensity in their vocalizations. The fossil record indicates that the first hominins with breath control like ours lived a maximum of 1.6 million years ago, which some suggest marks the first time our lineage would have been physically capable of producing vocalizations resembling singing (*Am J Phys Anthropol,* 109:341-63, 1999).

Genetics might also help researchers pin down when certain components of musicality appeared in our ancestors, if parts of our DNA can be linked to our capacity for perceiving and processing music. For now, however, the question of when humans first produced something we might recognize as music remains open to speculation.

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Why music?

As researchers continue to probe how humans have evolved to process music, many scientists, and the public, have been increasingly drawn to another question concerning musicality's origins: Why did it evolve at all? For some, music's universality in humans, combined with its fundamental social and cultural roles, is persuasive evidence that our musicality is adaptive. "Music is so common in all societies," says Helsinki's Järvelä. "There must be favorable alleles; it must be beneficial to humans."

But just what this benefit might be, and whether it did indeed influence our evolution, have been the objects of what Patel calls "one of the oldest debates in the book." In the late 1990s, cognitive psychologist Steven Pinker famously dubbed music "auditory cheesecake"—pleasant, but hardly essential—and argued that musicality was nothing more than a by-product of neural circuitry evolved to process language and other auditory inputs. It's become the argument to beat for researchers looking for ultimate explanations of musicality's evolution in humans, Fitch says. "Everybody seems to want to prove that Pinker's cheesecake argument is wrong," he notes. "But it's just the null hypothesis."

One adaptationist viewpoint, that traces its roots to Darwin, is that human musicality, like birdsong, is a sexually selected trait—albeit an unusual one, prevalent as it is in both sexes. Musicality is a reliable and visible indicator of cognitive ability, the argument goes, and so informs a potential mate of an individual's genetic quality. Some researchers have tried to generate testable predictions from this idea, but so far there's been little evidence in its favor. One recent study went as far as assessing the self-reported sexual success—based on indicators including the number of sex partners and age at first intercourse—of more than 10,000 pairs of Swedish twins.[10] The researchers found no association between musical ability and sexual success, but cautioned against being quick to draw conclusions about the sexual relationships of our evolutionary ancestors from modern society.

Other hypotheses arise from research on music's far more complex and still poorly understood effects on human emotion and social bonding. University of Toronto psychologist Sandra Trehub notes, for example, that babies and young children are particularly sensitive to musical communication, and that singing comes naturally to adults interacting with them. "Caregivers around the world sing to infants," she says. "It's not a Western phenomenon, nor a class-based phenomenon. It seems to be important for caregiving everywhere."

She and her colleagues recently showed that recordings of singing, more so than speech, could delay the time it took

Are musical tendencies the product of culture, or have they evolved along with our abilities to produce and process music?

for an infant to become distressed when unable to see another person.[11] And in 2014, research led by Laurel Trainor at McMaster University found that when babies just over a year old were bounced to music, they became more helpful towards a researcher standing opposite them who had been bopping along in rhythm (handing back "accidentally" dropped objects) than to people who had been bouncing asynchronously.[12]

These and related findings have led some to propose that parent-infant bonding, or social cohesion in general, provided a selective pressure that favored the evolution of musicality in early humans, though Trehub herself says she does not subscribe to this rather speculative view. "I have no difficulty imagining a time when music-like things would have been very important in communicating global notions and managing interpersonal relationships," she says. "But it's pretty hard, based on anything we look at now, to relate it to conditions in ancient times and the functions it would have served."

Indeed, the inherent challenge of studying ancient hominin behavior, combined with the complexity of the trait itself, makes explanations for musicality's evolution particularly vulnerable to "just-so" stories, says Trainor. "When you look at the effect that music has on people, it's easy to think it must have been an evolutionary adaption. Of course, it's very difficult, if not impossible, to prove that something is an evolutionary adaption."

This intractability has led some researchers to view adaptation-based lines of inquiry into human musicality as something of a distraction. "I don't think it's a particularly useful question at all," says Fitch. "It's an unhealthy preoccupation, given how little we know." Others have argued for a subtler view of musicality's evolution that avoids the search for simple answers. "The evolutionary process isn't a one-shot thing," says Trainor. "It has many nuanced stages."

Her work, for example, addresses how aspects of auditory scene analysis—the process by which animals locate the source of sounds in space—could have led to features currently viewed as critical for musicality in modern humans. But that doesn't mean that music didn't provide its own benefits once it arose. "I think parts of the long road to our becoming musical beings were driven by evolutionary pressures [for music itself]," says Trainor, "and other parts of it were driven by evolutionary pressures for things other than music that music now uses."

But most researchers agree that understanding our musical evolution will require studying musicality in more-focused and biologically relevant ways. For example, instead of asking why musicality evolved, Fitch suggests researchers investigate why humans evolved to synchronize their movements to a beat. This approach "is what's really important," says Patel. "We've had hundreds of years of speculation. Now, I think, the real advances are being made by thinking about the individual components of music cognition and looking at them in an evolutionary framework." ■

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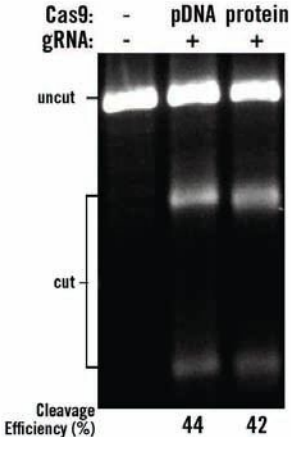

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The Animal Chorus

Many species are said to sing, but music is in the ear of the beholder.

BY *THE SCIENTIST* STAFF

For thousands of years, people have appreciated birdsong as one of nature's most melodic sounds. And for at least a few centuries, researchers have been talking about—and analyzing— birdsong, some attaching the label "music" to the avian behavior. In the mid-17th century, for example, German scholar Athanasius Kircher transcribed bird song with musical notation. Whether singing avian species hear their calls in a musical sense is, of course, anybody's guess. But still today, it's fairly uncontroversial to speak about bird vocalizations using terms such as "song" and "music."

Around the animal kingdom, several nonavians also produce sounds that are sometimes discussed using a musical vocabulary. Whale songs echo through the ocean for hundreds of miles, while frogs and crickets chorus on warm summer nights throughout much of the world. The stringency of the criteria for earning a label such as song varies by taxon, however. Birds, whales, mice, and even bats have a vocal repertoire that includes songs and simpler calls, while any insect or fish that produces sound for the sake of communication is considered, at least by some, to be "singing"—though no scientist seriously compares these species' chirps and grunts to birdsong.

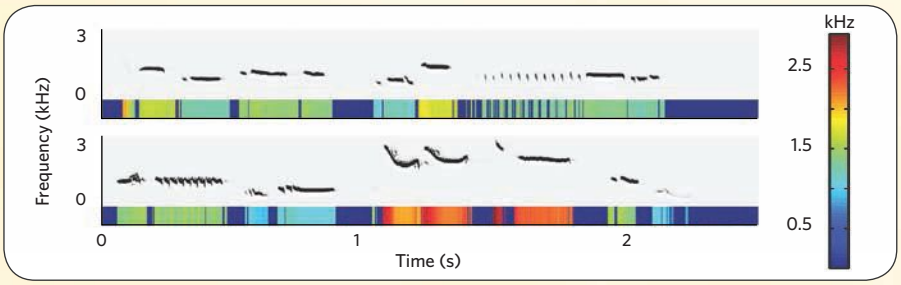
Semantics aside, more and more tonal or cadenced animal communication signals are attracting the attention of researchers. Technological advancements have enabled the study of mouse and bat calls that are broadcast in the ultrasonic range, as well as of the love songs of fruit flies, which vibrate their wings to produce sound within the frequency range of human hearing, but do so a million times more quietly than our ears can detect. And research continues to delve into the musical skills of diverse bird species that have long been recognized for their singing prowess, confirming that there is an overlap between the genes and brain areas involved in bird and human vocal learning.

Of course, "these systems are very different," notes Ani Patel, a psychologist Tufts University who studies music perception in people. "It's dangerous to go in thinking that animals do music the same way we do."

Tweety Tunes

To human ears, the trilling of birdsong ranks among nature's most musical sounds. That similarity to human music is now inspiring researchers to apply music theory to avian vocalizations. For example, zebra finch neurobiologist Ofer Tchernichovski of the City University of New York, together with musician and musicologist Hollis Taylor, recently analyzed the song of the Australian pied butcherbird (*Cracticus nigrogularis*) and found an inverse relationship between motif complexity and repetition that paralleled patterns found in human music (*R Soc Open Sci,* 3:160357, 2016).

Tchernichovski's work also suggests that birds can perceive rhythm and change their calls in response. Last year, he and colleague Eitan Globerson, a symphony conductor at the Jerusalem Academy of Music and Dance as well as a neurobiologist at Bar Ilan University in Israel, demonstrated that zebra finches, a vocal learning species, adapt their innate calls—as opposed to learned song—to avoid overlapping with unusual rhythmic patterns produced by a vocal robot (*Curr Biol,* 26:309-18, 2016). The researchers also found that both males and females use the brain's song system to do this, although females do not learn song.



A MUSICAL NUMBER: Like many avian species, the Australian pied butcherbird (right) produces a complex song (just two of five different phrases shown here) with patterns of repetition that resemble those in human music.

But these complexities of birdsong might be more comparable to human speech than to human music, says Henkjan Honing, a music cognition scientist at the University of Amsterdam. Honing's research suggests that some birds don't discern rhythm well. Zebra finches, for example, seem to pay attention to pauses between notes on short time scales but have trouble recognizing overarching rhythmic patterns—one of the key skills thought necessary for musical perception (*Front Psychol,* doi:10.3389/fpsyg.2016.00730, 2016).

Instead of rhythm, birds may be analyzing their peers' songs by the melody. A recent study found that European starlings (*Sturnus vulgaris*) distinguish shifting four-tone sequences by their "spectral shape," a statistical representation of all the frequencies in a sound most similar to timbre (*PNAS,* 113:1666-71, 2016). "They listen to sounds more as we listen to speech," Honing says.

But Tchernichovski contends it's unlikely that birdsong functions in this way. "Song is actually not language because the song is not information is a strict sense. It's much more like a performance," he says, where the goal is to captivate the audience.

When successful, a bird's song broadcasts individuality (and sometimes fitness) as well as membership in a species and even a local subculture, Tchernichovski says. In nature, male songbirds achieve this by embellishing the songs they learn from their fathers, but some distinct song characteristics appear to be at least partly innate. Zebra finches that learn from Bengal finches retain zebra finch temporal structure to their songs (*Science,* 354:1282-87, 2016), and Tchernichovski demonstrated that "self-tutored" zebra finches, which heard only playback of their own prerecorded melodies, developed and improved their songs just as quickly as juvenile birds who learned from adult males of their species (*Philos Trans R Soc Lond B Biol Sci*, 372:20160053, 2017). (See "Avian Accents" on page 49.)

How female birds recognize these cultural markers and decide which song is best "is a complete mystery," Tchernichovski says. That's partly because it's been difficult to design assays to measure which song elements females find the most appealing. Honing says a similar challenge faces researchers trying to assess the critical skills of song perception in birds.

Meanwhile, Honing argues that it says more about our brains than the birds' that we consider birdsong to be musical. "The proper and more informative question"—to which there is as yet no clear answer—"is, 'Is it music to them?'" **—Jenny Rood**

FROM *ROYAL SOCIETY OPEN SCIENCE*, 3:160357, 2016; © CHRIS WATSON/SHUTTERSTOCK.COM

Bat Broadcasts

In the past decade, some bat species have been added to the ranks of "singing" animals, with complex, mostly ultrasonic vocalizations that, when slowed down, rival the tunes of some songbirds. Like birds, bats broadcast chirps, warbles, and trills to attract mates and defend territories. There are about 1,300 known bat species, and the social vocalizations of about 50 have been studied. Of those, researchers have shown that about 20 species seem to be singing, with songs that are differentiated from simpler calls by both their structural complexity and their function.

Bats don't sound like birds to the naked ear; most singing species broadcast predominately in the ultrasonic range, undetectable by humans. And in contrast to the often lengthy songs of avian species, the flying mammals sing in repeated bursts of only a few hundred milliseconds. Researchers must first slow down the bat songs—so that their frequencies drop into the audible range—to hear the similarities. Kirsten Bohn, a behavioral biologist at Johns Hopkins University, first heard Brazilian free-tailed bats (*Tadarida brasiliensis*) sing more than 10 years ago, when she was a postdoc in the lab of Mike Smotherman at Texas A&M University. "I started hearing a couple of these songs slowed down," she recalls. "And it really was like, 'Holy moly—that's a song! That sounds like a bird.'"

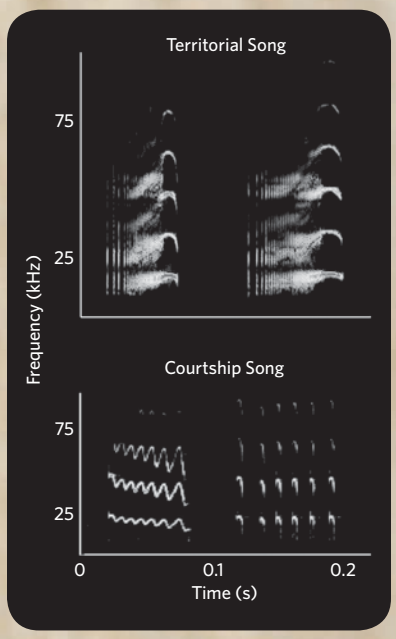
The neural circuitry used to learn and produce song may also share similarities between bats and birds. Bohn and Smotherman say they've gathered some tantalizing evidence that bats use some of the same brain regions—namely, the basal ganglia and prefrontal cortex—that birds rely upon to produce, process, and perhaps even learn songs. "We have an idea of how the neural circuits control vocalizing in the bats and how they might be adapted to produce song," Smotherman says.

In an exception to supersonic inaudibility, at least one bat species does sing loud and proud: the greater sac-winged bat (*Saccopteryx bilineata*), which broadcasts audible territorial defense songs from roosts tucked away in the canopies of tropical rainforests. "You can hear these songs from over a hundred meters away," says Mirjam Knörnschild, an animal behaviorist at the Free University of Berlin who studies the species in Costa Rica and Panama.

Greater sac-winged bats may even learn their songs in ways that mirror songbirds—and their pups "babble" the species' vocal repertoire as do songbird chicks and human babies, Knörnschild has shown (*Naturwissenschaften*, 93:451-54, 2006). "The species I work with happens to learn its territorial songs by imitating tutors, which is very close to what a bird is doing," she says. "There are fairly few species of bats well-described that sing, and even fewer that are capable of vocal learning. In that regard, *Saccopteryx* is the only one at present that is singing a song that is learned by vocal imitation."

The emerging similarities between bats' and birds' songs may be tied to the fact that both types of animals engage in one of the most resource-intensive modes of locomotion: flying. "You can use singing to control your territory without flying," which is energetically expensive, says Smotherman. "And singing works well to control fairly large territories for fairly small animals that fly. . . . There's an important tie between singing and flight."

—Bob Grant



HIGH-FREQUENCY FEATS: The greater sac-winged bat (above) sings to defend its territory (right, top) and to attract mates (bottom), among other things.

COURTESY OF MIRJAM KNÖRNSCHILD; © IAN BUTLER, COSTA RICA/ALAMY STOCK PHOTO

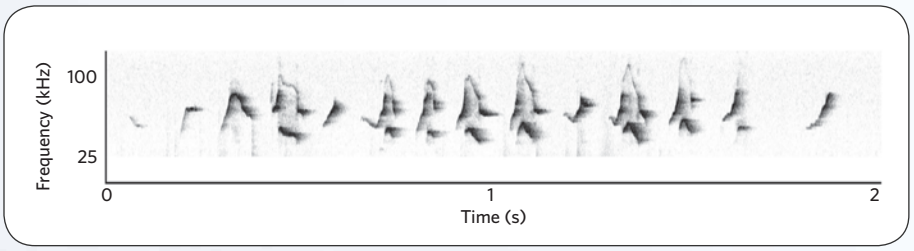
Silent Serenades

In 1877, Joseph Sidebotham, a Manchester cotton baron fascinated by natural history, published an informal correspondence in *Nature* describing how a mouse had serenaded him from the top of a woodpile. In the letter, Sidebotham notes that his son suggested that perhaps all mice can sing, but at frequencies that the human ear cannot hear, and that the audible mouse vocalist was an oddity (what today we would call a mutant).

Sidebotham dismissed his son's idea, but it turned out to be right: mice do sing. In addition to the audible squeaks for which they are known, the rodents produce more-elaborate vocalizations reminiscent of birdsong, but at a frequency far beyond the limits of human hearing. In 2005, Timothy Holy, a neuroscientist at Washington University in St. Louis, and colleagues defined these ultrasonic vocalizations as songs using measures similar to those that researchers employ to distinguish songs from isolated calls in birds (*PLOS Biol*, 3:e386). "It was really when I wrote an algorithm that allowed me to shift the pitch of these calls that the analogy to bird songs became apparent," Holy says. He described the mouse high-frequency vocalizations as "distinct syllable types uttered in sequences, and some sort of temporal patterning," just like bird songs. Building on Holy's work, neurobiologist Erich Jarvis and colleagues found that male mice sing complex ultrasonic songs to attract mates. (See "Singing in the Brain" on page 50.)

"There's acoustic structure to the vocalizations mice emit, and they are used during social interactions," says Roian Egnor, who studies the neural basis of vocal behavior in mice at the Janelia Research Campus in Virginia. "But," she adds, "whether it's music is way above my pay grade."

The supersonic songs of lab mice are critical for a normal social life. In 1983, Steven Pomerantz, then at Michigan State University, demonstrated that female mice spend more time with males who can sing than devocalized animals. More recent studies have demonstrated that dominant male mice that mate more also sing more, while subordinate males mate and sing less (*Front Neurosci*, 8:231, 2014). "There's something that happens when you become a subordinate male that suppresses your ability or interest in singing," Egnor says.



SILENT SONGS: The ultrasonic calls of rodents are important for mediating social interactions among the animals.

Indeed, male singing behavior in diverse species, including mice, has been linked to testosterone levels, which tend to increase with dominance and decline with subordination.

Beyond the male's courtship song, female mice appear to use their own repertoire of ultrasonic calls to celebrate reunions with littermates or to settle territorial disputes. So both male and female mice sing in response to females, says Egnor, "but if you put a male with another male, you get no ultrasonic vocalizations at all. They don't say anything—they just fight."

Mice are not alone; rats, squirrels, and hamsters also emit supersonic vocalizations. How rodents produce these ultrasonic vocalizations, which clock in at frequencies above 50 kHz in lab mice, remains unclear. Unlike bats, for instance, which evolved muscular larynxes to produce high-frequency echolocation vocalizations, mice manage to produce supersonic songs with woefully average vocal cords.

Neuroscientists are also beginning to explore the genetic and neurologic components of rodent vocalizations, which could have implications for humans as well, says Terra Barnes, a postdoc at Washington University in St Louis. "At least some of the neurons necessary to make vocalizations involve a neural pathway that is similar between mice and humans." In 2016, Barnes and colleagues demonstrated that mice develop vocalization problems when engineered to carry a mutation homologous to one responsible for some types of human stuttering (*Curr Biol*, 26:1009-18).

"We just finished characterizing the vocalizations of the mouse model," Barnes says. "Now we get to find the neural mechanism, and look for potential therapies."

—Joshua A. Krisch

FRONT BEHAV NEUROSCI, 10:197, 2016; © JNE VALOKUVAUS/SHUTTERSTOCK.COM

Fields of Frogs

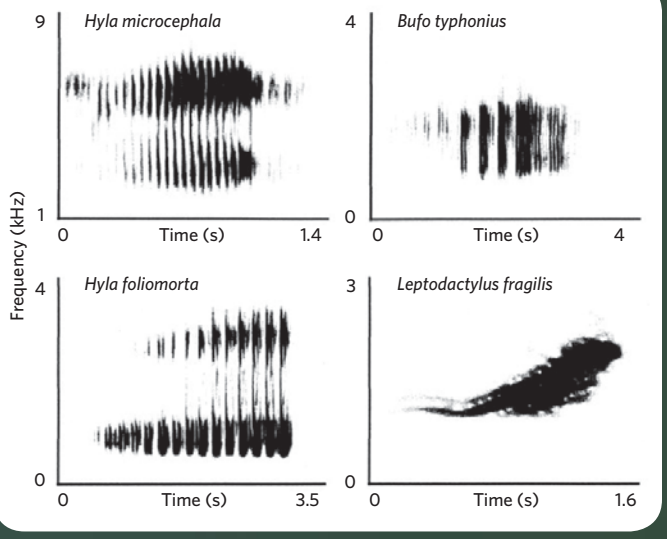
In anurans—the group of tailless amphibians to which frogs and toads belong—vocalization is all about sex. Males produce the majority of these sounds, most often to attract mates and defend territories. Many species have vocal sacs that amplify these so-called advertisement calls, which vary widely. "Every species has [its] own unique call," says population geneticist Benjamin Pierce of Southwestern University in Georgetown, Texas. "Some are grunts, some are trills, some are peeping noises."

Despite the differences in vocalizations among frogs and toads, the repetitive nature of the calls is seen across diverse species. "Many of them call at—more or less—a fixed rhythm," says zoologist Mike Ryan of the University of Texas at Austin. And heard in groups, he adds, "a lot of them sound very musical."

Frogs rarely call in isolation. Males typically broadcast amongst a gaggle of competitors, and across the population, vocalizations overlap and syncopate—the animals are said to be chorusing. "Listening to a chorus of frog calls, it definitely has rhythm—it builds, it dies down—and it certainly can be 'music,'" says Carlos Davidson, a professor of environmental studies at San Francisco State University.

"There's melody, harmony, and repetition in frog calls," agrees Phil Bishop, who studies amphibian communication at the University of Otago in New Zealand.

And the synchronization of the anuran chorus is not totally random. To attract the attention of potential mates in a competitive environment, male bird-voiced tree frogs (*Hyla avivoca*) adjust their pulse rates (*Behav Ecol Sociobiol,* 63:195-208, 2008). Female grey tree frogs (*H. versicolor*) showed a preference for leading pulses when researchers played a pair of overlapping pulsed signals that were typical of males of this species (*Anim Behav,* 80:139-45, 2010). And the calls of large groups of frogs are more likely to reach potential mates than the sounds of a lone amphibian, Davidson notes. While "each individual is calling for [his] sole benefit," he says, "females will be more likely to hear and be attracted to . . . males all calling together."



A DIVERSITY OF CHORUSES: Dozens of frog species call for mates. Vocalizations range from low frequency grunts to broad-spectrum trills. (Call of *Hyla versicolor*, pictured at left, is not shown.)

Davidson is also interested in creatures that eavesdrop on frog calls, such as nearby predators that might use the sound to deduce the size and location of potential prey. "There may be audiences—other animals that hear the sound—for which it has a very different meaning," he says.

There are also human audiences. Pierce and colleagues survey frog calls in central Texas in order to identify which—and how many—frogs are present in a given environment. "You can [identify] about 90 percent of the species that are calling in 15 minutes," Pierce says. "If you listen for 5 minutes or 10 minutes, you're likely to miss some things."

—Tracy Vence

BEHAV ECOL, 11:71-83, 2000; © ISTOCK.COM/CATHYKEIFER

Insect Complexity

Contrary to the din of some warm summer nights, with choruses of grasshoppers, katydids, crickets, or cicadas chirping away, relatively few insects use acoustic information to communicate with their peers. In fact, outside of these familiar groups, only a very few moth, butterfly, and fly species produce calls. For the insects that do, however, researchers say that sounds they make constitute "singing."

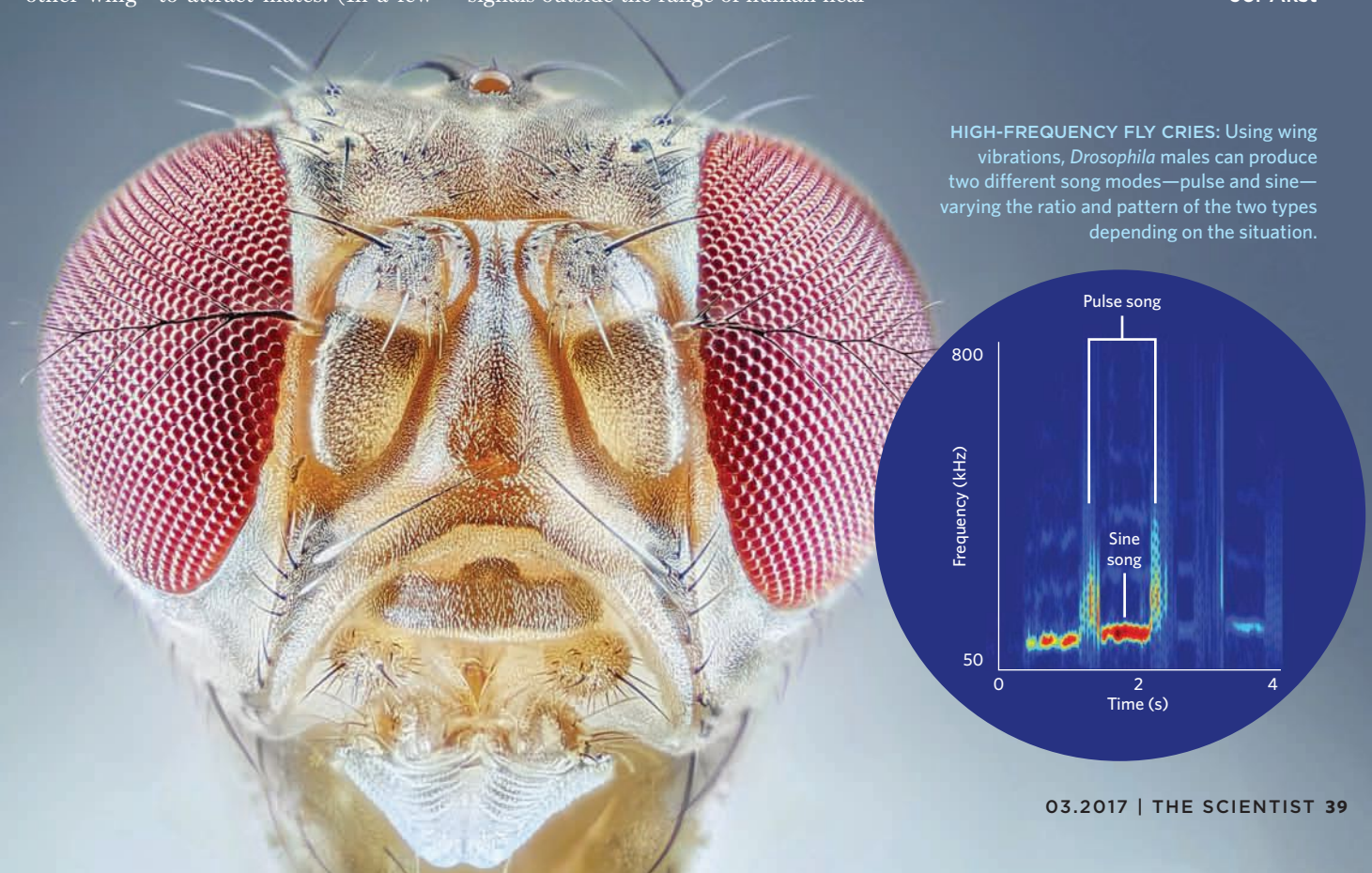
"I don't want to get caught up in semantics, but it basically has to do with using sound as a means of sending information," says evolutionary biologist Michaël Greenfield of CNRS's insect research institute (Institut de Recherche sur la Biologie de l'Insecte) at the University of Tours in France. "If they make a sound, we call it a song."

Most insect sounds are relatively simple. In crickets and related species, for example, males repeatedly produce a simple chirp—made by rubbing the top edge of one wing across serrations on the other wing—to attract mates. (In a few species, females are known to duet with males.) But a group of crickets chirping in the same vicinity can produce something quite complex indeed. As in frogs and toads, the sum of the population's calls is termed a chorus, as the calls take on "a certain harmony," says Greenfield. But to date, there is no evidence that this chorusing serves any function in insects. In fact, Greenfield and his colleagues have recently shown in one species of katydid, *Ephippiger diurnus,* that chorusing is an emergent property of the insects' tendency to adjust the timing of their calls to avoid singing just after a neighboring male, as females tend to ignore calls that follow too closely behind another (*Behav Ecol Sociobiol,* 69:1957-73, 2015; *Sci Rep,* 6:34369, 2016). "When everyone is playing that game, trying to time their song effectively, then the chorus emerges," says Greenfield.

The calls of other singing insects are undetectable by the human ear. Moths, for example, broadcast ultrasonic mating signals outside the range of human hearing, and *Drosophila* sing very quietly—at least a million times below the human threshold. First documented in the 1950s, *Drosophila* "song" is actually quite complex relative to the calls of other insects, says Pip Coen, a research associate at University College London. In contrast to the simple cricket chirp, *Drosophila* calls, produced by wing vibrations, have two modes—pulse and sine—and a male can vary which he produces depending on the situation: courtship of females versus conflict among males, for instance.

The intricacy is interesting in and of itself, but also for what it says about *Drosophila* neurobiology. "It's a complex signal the brain has to interpret," says Coen—and only in the last decade or so have researchers been able to record directly from the central brain neurons in female flies to start to assess the neural circuitry underlying the perception and processing of males' calls. "We're just realizing how interesting and complex a problem it is."

—Jef Akst



HIGH-FREQUENCY FLY CRIES: Using wing vibrations, *Drosophila* males can produce two different song modes—pulse and sine—varying the ratio and pattern of the two types depending on the situation.

COURTESY OF PIP COEN; © SEBASTIAN JANICKI/SHUTTERSTOCK.COM

Big Voices

In a 1971 paper published in *Science*, biologist Roger Payne, then at Rockefeller University, and Scott McVay, then an administrator at Princeton University, described the "surprisingly beautiful sounds" made by humpback whales (*Megaptera novaeangliae*; *Science*, 173:585-97). Analyzing underwater recordings made by a Navy engineer, the duo found that these whale sounds were intricately repetitive. "Because one of the characteristics of bird songs is that they are fixed patterns of sounds that are repeated, we call the fixed patterns of humpback sounds 'songs,'" they wrote.

It's now clear that, in addition to simpler calls, several baleen whale species—including blue, fin, and bowhead—make series of sounds known as song. Humpback song is the most complex and by far the best studied. Units of humpback songs form phrases, series of similar phrases form themes, and multiple themes form songs. All the males in a given population sing the same song, which evolves over time. When whale groups come into contact, songs can spread. (See "Peter Tyack: Marine Mammal Communications," *The Scientist*, June 2016.)

But why do whales sing? "The short answer is, we don't know," says Alison Stimpert, a bioacoustician at Moss Landing Marine Laboratories in California. Humpback songs are only performed by males and are often heard on breeding grounds, so the dominant hypothesis is that these songs are a form of courtship. The quality of a male's performance could be a sign of his fitness, for example. But female whales do not tend to approach singing males. Alternatively, whale researchers have proposed that the male whales sing to demarcate territory or to form alliances with other males during mating season.

Song may have a function outside of the breeding season as well. Stimpert, for instance, has recorded humpback song on an Antarctic feeding ground, sometimes in areas where the whales were lunging for food (*PLOS ONE*, 7:e51214, 2012).

Eduardo Mercado III, a professor of psychology at the University at Buffalo in New York, says that labeling patterned whale calls "song" led researchers to focus closely on reproduction. "It gave people tunnel vision about how you should study song and what you should look for," he says. Mercado proposes that humpback song could be a form of echolocation, in which whales listen to the echoes of their own and other whales' vocalizations—which can travel multiple kilometers through the water—to navigate the ocean and detect large masses, like other whales, from far away.

As debate continues on the function of humpback song, researchers continue to catalog the sounds of other marine species. In September 2016, for example, biologists described an unfamiliar sound—hypothesized to be a minke whale call—in the area of the Mariana Trench. "There's still new stuff out there, and we're still figuring out what produces it," says Stimpert.

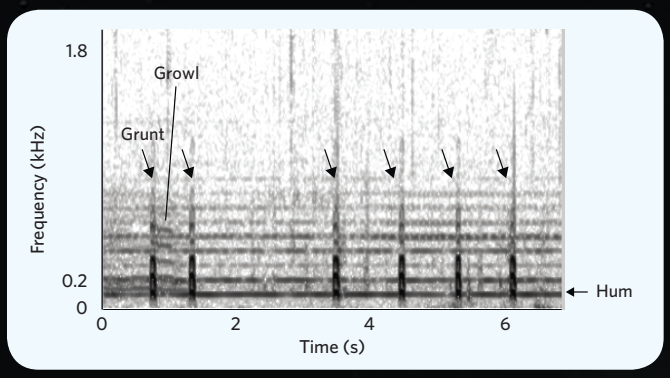
—Kate Yandell

OCEAN SONGS: Humpback whales make diverse, broadband sounds that travel miles through the ocean. Their function, however, remains somewhat murky.

PLOS ONE, DX.DOI.ORG/10.1371/JOURNAL.PONE.0079422, 2013; © ISTOCK.COM/PAUL WOLF

The Ocean's Drummers

In 1986, neuroscientist Andrew Bass had just landed a job as an assistant professor at Cornell University when he decided to pay a visit to the Steinhart Aquarium in San Francisco to see the facility's plainfin midshipman (*Porichthys notatus*), a foot-long, wide-lipped toadfish that lives along the coast of California. But it turned out he was in for something even better than watching the fish swim on the other side of aquarium glass. A staff member at the aquarium knew of midshipman nests in Tomales Bay, a little more than an hour's drive north of the city. So Bass got in the car to see the animals in their natural habitat.



FISH TALK: Midshipman fish produce a few different calls, including grunts, growls, and hums.

As Bass stood on the rocky shore that evening, he heard a low rumble emanating from the water. It was male fish, humming, doing their best to lure females to their dens. "I'll never forget it," he says. "They were chorusing!"

Since that California night 30 years ago, Bass has revealed the intricacies of the midshipman's calls—territorial grunts and growls in addition to mating hums—and the corresponding anatomy, neural circuitry, and hormonal control that governs the acoustic behavior. Just a few months ago, for instance, he figured out that melatonin in the sound-production regions of the midshipman brain is responsible for maintaining a circadian rhythm to the fish's singing (*Curr Biol,* 26:2681-89, 2016).

While the midshipman's calls may arguably be the most interrogated of the fish world, the species is by no means the only noisy fish in the sea. Myriad fish species have come up with a variety of ways to make sounds, from contracting muscles around their swim bladders (as toadfish such as the midshipman do) to wiggling their pectoral fins (as catfish do) to grinding teeth (as parrotfish do) or rubbing together bones in their heads to make a clicking sound (as seahorses do). "Most people are not aware fish make songs," says Bass. "They're the frogs of the ocean."

In addition to attracting mates and defending territory, there's recent evidence that fish may also use sound as a contact call—letting conspecifics know where they are. Craig Radford, a fish biologist at the University of Auckland, showed that captive bigeyes (*Pempheris adspersa*) clustered together when they were played conspecific calls, and also swam closer to one another when background reef noise was made louder (*Sci Rep,* 6:19098, 2016). "They use a click sound to help maintain school structure," Radford says, similar to the way elephants project a low rumble over long distances to check in with one another.

Whether the underwater cacophony constitutes music is up for debate. Radford doesn't describe fish sounds as music, and Friedrich Ladich, who studies acoustic communication among fish at the University of Vienna, agrees. "I think it's an exaggeration to call fish sounds songs," he says. But other researchers do describe the animals as singing. Andrew Bass is one of them. "To me, I've interpreted song as an advertising signal produced in a reproductive context," Bass says. And at least in the case of midshipman fish, this definition most certainly applies, he adds. When the males' hums are projected underwater, "females make a beeline for the speaker. . . . People think of it as a love song."

—Kerry Grens

ANDREW HOWARD BASS, *J EXP BIOL*, 217:2377-89, 2014; © DAVID WROBEL/GETTY IMAGES

Music Therapy

The principles of neuroplasticity may underlie the positive effects of music therapy in treating a number of diseases.

BY ELIZABETH STEGEMÖLLER

A man with Parkinson's disease sitting in a crowded restaurant has to use the rest room, but he cannot get there. His feet are frozen; he cannot move. The more he tries, the more stressed he becomes. People are beginning to stare at him and wonder what is wrong. Then he remembers the song "You Are My Sunshine," which his music therapist taught him to use in situations like this. He starts humming the tune. In time with the music, he steps forward—one foot and then the other—and begins walking to the beat in his head. Still humming, he makes it to the rest room, avoiding a potentially embarrassing situation.

Freezing of gait is a common occurrence for many people with Parkinson's disease. Such struggles can limit social experience and lead to seclusion and depression. Unfortunately, available pharmacological and surgical treatments for Parkinson's do a poor job of quelling this and many other symptoms. But where conventional medicine has failed, music therapy can sometimes provide relief.

Music therapy is the use of music by a credentialed professional as an intervention to improve, restore, or maintain a non-music-related behavior in a patient or client. As a music therapist, I have worked with many people with Parkinson's disease and have seen how music can provide an external cue for patients to walk in time to, allowing them to overcome freezing. I have also used group singing to help patients with Parkinson's improve their respiratory control and swallowing. Impaired swallowing can lead to aspiration pneumonia, which is a leading cause of death among this patient population.

But perhaps the most powerful component of music therapy is the social benefit derived from making music together, which can help patients combat depression. When patients with Parkinson's engage in music therapy, often one of the first behaviors to emerge is smiling, and the flat affect and masked face that are characteristic symptoms of the disease fade away. These participants comment on how music therapy is the best part of their week, and their caregivers state that their loved ones are in much

better moods—with fewer Parkinson's symptoms—after returning home from music therapy.

While all of this is interesting, it is not new. Aristotle and Plato were among the first to write on the healing influence of music. The earliest references to music as therapy occurred in the late 1700s and early 1800s, and the field formally began after World War I, when professional and amateur musicians played for veterans who had suffered physical and emotional trauma as a result of the war. Nowadays, certified music therapists seek to do more than just play the right song at the right time. They use music to help people with many different physical and emotional disorders or diseases.

A large body of research focused on quantifying changes in behavior has provided strong evidence that music therapy is a powerful intervention for a variety of patients or clients. (See table on page 47.) Nevertheless, it is still not always included in standard medical practice, and there is limited to no reimbursement from insurance companies for music therapy services. This resistance stems, in part, from a major unanswered question: How does music therapy work?

The diversity of patient populations and music therapy settings, as well as the multifaceted features of music itself, make understanding how the brain changes in response to this intervention very difficult. As a result, few researchers have even attempted to delve into the neuroscience of music therapy. However, I believe the answer may be right in front of us—in existing literature on neuroplasticity. By pulling together what we know about how the brain works—independently of how it responds to music therapy—I believe researchers can generate a mechanistic framework on which current and future research in music therapy can be built.

Answers in neuroplasticity

Neuroplasticity is the ability of the brain to change throughout a person's life span as a consequence of sensory input, motor action, reward, or awareness. American psychologist and physician William James first noted in the late 1800s that people's behaviors were not static over time, and not long after, Spanish neuroscientist Santiago Ramón y Cajal suggested that behavioral changes had an anatomical basis in the brain. It is now well accepted that neuroplasticity encompasses changes on multiple levels, from individual synapses to entire cortical networks.

The goal of music therapy is to elicit behavioral changes in a patient, and these changes are likely underpinned by changes in the brain. Indeed, I argue that three simple principles of neuroplasticity may explain how music therapy works. (See illustration below.)

The first has to do with the brain's reward circuitry. Research has consistently shown that dopamine is a primary neurotransmitter involved in neuroplasticity, and dopaminergic neurons in the reward network of the brain, including the ventral tegmental area (VTA) and nucleus accumbens (NA), have been implicated in cortical remodeling, reward-related learning, and hippocampal long-term potentiation (the strengthening of synapses due to a

MECHANISMS OF MUSIC THERAPY

Music therapy has been successful in treating a variety of diseases and disorders, but to date, little is known about the mechanisms underlying its effects. Looking to the literature on neuroplasticity—the ability of neurons to change their connections and networks—could provide some answers.

REWARD

Enjoyable music activates the reward network of the brain, including the ventral tegmental area (VTA) and nucleus accumbens (NA). By pairing such rewarding music with desired, nonmusic behaviors, music therapists may be tapping into the brain's reward pathway.

long-lasting increase in signal transmission between two neurons). And in the past few years, researchers have demonstrated that, like food and drugs, enjoyable music activates these reward networks.[1,2] Thus, by pairing music with non-music-related behaviors, music therapists may be tapping into the brain's reward pathway.

Three simple principles of neuroplasticity may explain how music therapy works.

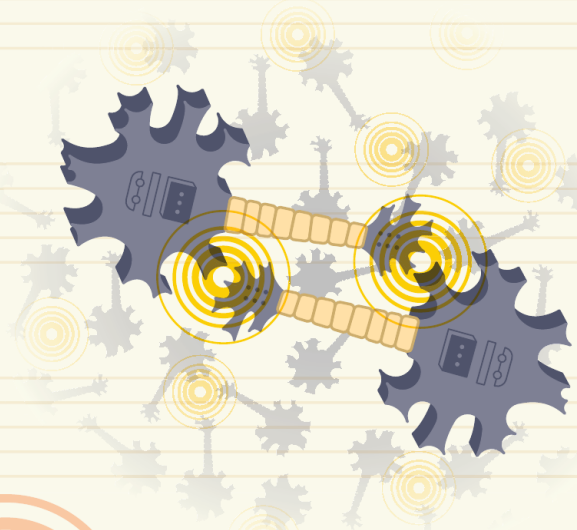
In 2001, Harvard University's Anne Blood and colleagues reported that cerebral blood flow in brain regions associated with reward changes in response to music that elicits "chills."[3] Subsequent imaging studies have revealed that the VTA and NA are activated when listening to enjoyable music (study participants are usually asked to bring in their own preferred music), and that activation of the NA predicts how positively a participant will rate the experience of listening to a piece of music.[4,5] Another group found that dopamine is released in these same reward centers in anticipation and experience of peak emotion in music.[6]

When I work with patients with Parkinson's disease to overcome freezing of gait, I pair music with walking. When I work with patients to strengthen respiratory control, I sychronize music with inspiration and expiration. Because I use a patient's preferred music—standard practice in music therapy—the music is by definition enjoyable. I hypothesize that this activates the brain's reward networks and helps reinforce the learning of non-music-related behaviors, such as walking or respiratory control.

A second principle in neuroplasticity is the Hebbian theory, introduced in the middle of the 20th century by Donald Hebb and summarized by neuroscientist Siegrid Löwel of the University of Göttingen as "Neurons that fire together, wire together." In other words, for two neurons to make a new connection or strengthen an existing one, they must fire action potentials synchronously. Research has shown that sensory stimuli can cause neural populations to fire synchronously. Rhythm, for example, is an inherent feature of music that, in addition to linking diverse behaviors to an external beat (a phenomenon known as entrainment), may also induce synchrony in the neural networks underlying the behaviors. (See "Music from Mayhem" on page 49.) Thus, by pairing music with activities such as movement, vocalization, breathing, and heart rate, music therapists may be eliciting simultaneous firing of neurons in brain areas involved in the control of those behaviors, strength-

THE HEBBIAN THEORY
Neurons that fire simultaneously make stronger connections. The rhythm of music played during a therapy session may be eliciting such neuronal synchronization at the same time that it helps patients regulate their movement, vocalization, breathing, or heart rate.

NOISE
Exposure to noise can be stressful and can impair cognition and memory. But music is essentially the opposite of noise, with high levels of consonance. Thus, music therapy may provide a clear acoustic signal to help patients learn desired non-music-related behaviors.



ening neuronal connectivity and leading to faster and more-permanent changes in their patients.[7]

Conversely, researchers have shown that noise—disordered sound that is meaningless and tends to be unpleasant—can have negative impacts on neuroplasticity. Research in animal models has shown that exposure to noise can induce stress and impair both cognition and memory by suppressing long-term potentiation in the hippocampus. Moreover, in rodent models, researchers have shown that embryos exposed to noise experience changes in the auditory cortex, hippocampus, and limbic system, and these changes may lead to decreased memory function and anxiety. Exposure to music, on the other hand, can promote neuroplasticity. Long-term prenatal exposure to music improves spatial learning due to changes in hippocampal function in rats.[8] In human subject research, extensive music training and experience also leads to brain changes in areas involved in auditory and motor processing.[9,10,11]

Perhaps music's strongest feature is that it interacts with diverse regions of the brain.

The acoustic structure of music may explain the underlying differences in the impact of noise versus music on neuroplasticity. One could consider music to be the polar opposite of noise. My own research, examining the acoustic structure of song, revealed that song is more consonant than speech. My group found that professionally trained musicians have less "noise" in both their spoken and sung acoustic signals. Music therapists are professionally educated musicians with training in many instruments and voice, enabling them to minimize the amount of noise and optimize the resonating precision of their musical sounds. Thus, music therapists may provide clearer acoustic signals, whether instrumental or vocal, than other clinicians, thereby promoting neuroplasticity in the brains of the patients they treat.[12]

Although researchers have yet to demonstrate a direct link between music therapy's effects and neuroplastic changes in the brain, it's interesting to note that neuroplasticity is typically not lost with disease or injury. But neuroplasticity can be maladaptive, such as in the case of stroke, when changes to the brain are often pathological. Many researchers are working to better understand how to suppress neural changes associated with undesirable behaviors while promoting changes that are beneficial. Perhaps, in addition to the neuroplasticity literature informing the mechanisms of music therapy, a better understanding how music therapy leads to positive changes in behavior across diverse patient populations will provide insight into these neuroplasticity processes.

Music therapy in action

Music therapists work with a wide range of patients and clients to change many behaviors not related to music. (See table on opposite page.) These include patients going through painful procedures and/or trauma, for whom music can reduce pain, fatigue, and anxiety, perhaps decreasing the amount of sedation and analgesia needed. I have witnessed a therapist entraining a song's rhythm to a distressed patient's heart rate, then systematically slowing the rate of the music to slow the patient's heart rate and, in turn, calm the patient. I have also witnessed music therapists modulate respiration rate through entrainment leading to greater relaxation and less anxiety.

Music therapists can also play an integral part in helping children with special needs by using music to increase vocalization/verbalization to improve effective communication. I have worked with a nonverbal boy with autism, for example. While playing his favorite song as he kept time on the drum, I left out the last word of a phrase and had him fill in the gap—the first time he had ever verbalized a word. From this point forward, the boy began to speak more words and communicate more effectively with his mom. As in the case of the patient with Parkinson's disease, using client-preferred music and rhythm with a clear music signal may have resulted in synchronous firing of the circuitry underlying verbalization, cre-

ating new connections that were strengthened through dopaminergic mechanisms.

Modulating dopamine, synchronizing neural activity, and reducing noise can all promote neuroplasticity. Music therapy capitalizes on all three of these principles, and I believe it is the only therapy that can. Music therapists use music to increase activity in dopamine-related reward networks. They use rhythm to synchronize neural activity of non-music behaviors. And they are trained musicians with reduced noise in their acoustic signals.

Perhaps music's strongest feature, though, is that it interacts with diverse regions of the brain. Music can activate brain regions involved in listening to, reading, moving to, and playing music, and in the experiencing of memories, emotional context, and expectations associated with music. Music therapists manipulate music to bring about a desired change in non-music-related behavior, specifically targeting brain regions underlying these behaviors.

With the emergence of new neuroimaging technologies and a focus on neuroscience research in health, music therapy is primed for continued investigations and new findings. The better we can understand the neural mechanisms underlying its effectiveness, the more music therapy can reach those in need. ■

Elizabeth Stegemöller is a board-certified music therapist and neuroscientist at Iowa State University, where she studies the effects of music on movement and associated neurophysiology in persons with Parkinson's disease.

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MUSIC THERAPY IN ACTION

Music has shown positive effects in a variety of patient populations for improving symptoms related to different diseases and disorders. Here's a sampling of some of the more common uses of music therapy.

Patient population	Nonmusic behaviors
Autism spectrum disorder	Movement, communication, speech and language, social skills, attention, cognition, activities of daily living
Alzheimer's disease and dementia	Memory, mood, social interaction
Traumatic brain injury	Movement, communication, speech and language, social skills, attention, memory, cognition
Mental health and mood disorders	Self-esteem, awareness of self and environment, expression, reality testing, social skills, attention, cognition
Pain management	Anxiety and stress, mood, feelings of control
Cancer	Anxiety and stress, mood, feelings of control, coping skills
Movement disorders and stroke	Movement, speech and language, swallowing , respiratory control, memory, cognition
Hospice	Anxiety and stress, mood, feelings of control, coping skills

The Literature

EDITOR'S CHOICE IN NEUROSCIENCE

Taking Notes

THE PAPER

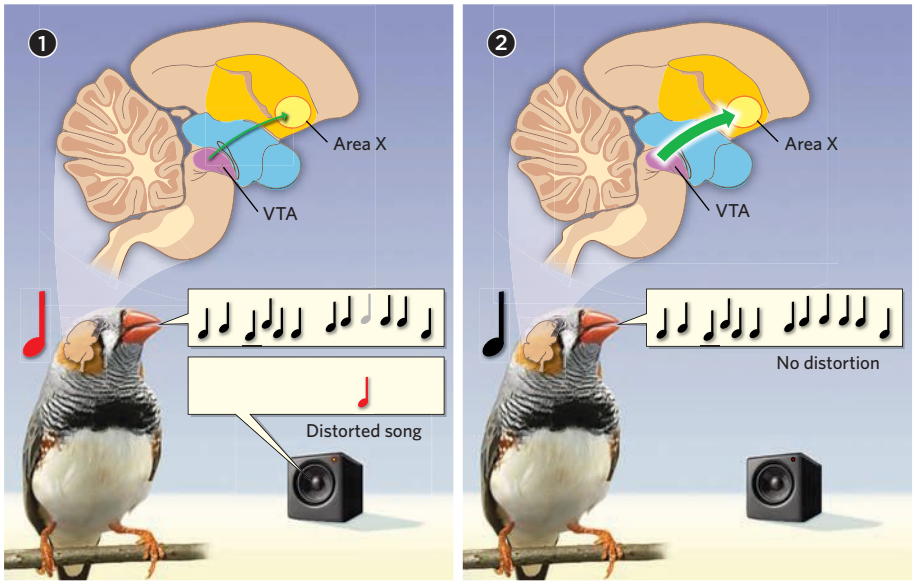
V. Gadagkar et al., "Dopamine neurons encode performance error in singing birds," *Science*, 354:1278-82, 2016.

Recognizing when you're singing the right notes is a crucial skill for learning a melody, whether you're a human practicing an aria or a bird rehearsing a courtship song. But just how the brain executes this sort of trial-and-error learning, which involves comparing performances to an internal template, is still something of a mystery. "It's been an important question in the field for a long time," says Vikram Gadagkar, a postdoctoral neurobiologist in Jesse Goldberg's lab at Cornell University. "But nobody's been able to find out how this actually happens."

Gadagkar suspected, as others had hypothesized, that internally driven learning might rely on neural mechanisms similar to traditional reward learning, in which an animal learns to anticipate a treat based on a particular stimulus. When an unexpected outcome occurs (such as receiving no treat when one was expected), the brain takes note via changes in dopamine signaling.

So Gadagkar and his colleagues investigated dopamine signaling in a go-to system for studying vocal learning, male zebra finches. First, the researchers used electrodes to record the activity of dopaminergic neurons in the ventral tegmental area (VTA), a brain region important in reward learning. Then, to mimic singing errors, they used custom-written software to play over, and thus distort, certain syllables of that finch's courtship song while the bird practiced. "Let's say the bird's song is ABCD," says Gadagkar. "We distort one syllable, so it sounds like something between ABCD and ABCB."

The team found that birds who listened to songs with the distorted syllable had less neural activity among dopamine-producing neurons in the bird's brain. "That's exactly what we were looking for," says Gadagkar. "There's a signal in the VTA saying, 'Ah, this is not what I wanted to hear; the song is not right.'"



NEGATIVE FEEDBACK: In the brain of male zebra finches, dopaminergic neurons (green arrows) project from the ventral tegmental area (VTA) to Area X, a region known to be required for song learning. Researchers from Cornell University found that these neurons encode singing errors by suppressing dopamine signaling when the bird hears itself producing an incorrect note, which researchers simulated by the introduction of distorted audio feedback at specific syllables ❶—and boosting dopamine signaling when the bird correctly produces a note that sounded incorrect in previous attempts ❷.

Although the observations reveal correlation, not causation, between singing errors and dopamine signaling, Gadagkar's work provides "powerful" support for the idea that dopaminergic neurons help mediate internally driven learning, says Richard Mooney, a neurobiologist at Duke University. "It's an important result," he says. "In some sense, it's expected, but this is one of these cases where confirming a long-standing prediction is really valuable."

The researchers also found something they weren't looking for. After many trials, the zebra finches began to respond differently to their own songs: when the birds heard a correct syllable that had been distorted in previous trials, VTA neurons showed higher dopamine activity than normal. "We interpret that as a 'better-than-expected' signal," Gadagkar explains. "This means that the birds are not just comparing what they sang to the template. They're also comparing it to their recent practice."

This finding is particularly intriguing and deserves further study, notes Mooney. "I'm dying to know what the time [aspect] of this memory function is," he says.

Gadagkar is now exploring dopamine-based song evaluation in the presence of the bird's intended audience: a female. "These birds sing in two modes, practice and performance," he says. "When he's singing to a female, it's game on. So what happens when you sing to a female and make a mistake?" —Catherine Offord

BEAT IT: In a lab experiment based on the "telephone" game, rhythm evolved from random tempos.

VOCAL COACH: A zebra finch (left) learned to sing from its foster parent, a Bengalese finch (right).

BEHAVIOR

Music From Mayhem

THE PAPER

A. Ravignani et al., "Musical evolution in the lab exhibits rhythmic universals," *Nat Hum Behav*, 1:0007, 2016.

MUSICAL UNIVERSALS

Although Beethoven's orchestral symphonies may contrast with the synthetic sounds of today's electronic beats, music from different genres has a lot in common. In 2015, a group led by Patrick Savage of Tokyo University of the Arts found 18 musical features that consistently appeared across geographical regions.

"BROKEN TELEPHONE"

Six of the features were related to rhythm, and Andrea Ravignani, a postdoctoral researcher at Vrije Universiteit Brussel in Belgium, and his colleagues decided to see whether these would spontaneously emerge in the lab. They gathered 48 non-musicians to play a modified version of the "telephone" game. In groups of eight, the subjects each sequentially played their best imitation of a randomly generated drumming sequence. By the time the musical message made its way to the end, it had transformed into a predictable pattern. "We could see that [the rhythms] became more regular, more structured, more organized, easier to imitate, and converged toward all these six rhythmic universals found in world music," Ravignani says.

CROSS-CULTURE CONVERGENCE

"I think this is an elegant study in its design, [which] enables a very strong demonstration of these preferences that the brain has," says Guy Madison of Umeå University in Sweden who was not involved in the study.

INNATE BIASES

Ravignani believes that these stereotypical patterns may arise because of biological constraints such as limited working-memory capacity, though this has to be investigated further. He hopes to take this experiment around the world to see whether these universals emerge in other cultures, particularly in places like the Balkans, where traditional music is based on much more complex rhythmic patterns.

—Diana Kwon

NEUROSCIENCE

Avian Accents

THE PAPER

M. Araki et al., "Mind the gap: Neural coding of species identity in birdsong prosody," *Science*, 354:1282-87, 2016.

VOCAL SIGNATURES

Bird songs contain scads of information, including a bird's species identity and reproductive potential. According to Yoko Yazaki-Sugiyama of the Okinawa Institute of Science and Technology, there are two competing elements within an individual bird's song: the characteristics common to all members of their species, and distinct elements that each male bird develops.

FOSTER PARENTS

How zebra finches retain species identity within their unique songs was not clear, so to find out, Yazaki-Sugiyama and her team placed baby zebra finches with Bengalese finch parents. Although the young zebra finches adopted the syllables of their foster parents' songs, their tunes maintained their own species-specific rhythm, the researchers found, coded as silent gaps between sounds. Yet the birds had never heard their conspecifics sing.

NEURAL BARCODES

To uncover the neural origins of this innate "accent," the team recorded neurons from the auditory cortices of birds listening to birdsongs from both their own and other species and found two different populations of neurons—one activated during the learned syllables and the other during the silent gaps. "Having the two independent systems is a big benefit for overcoming two competing criteria," says Yazaki-Sugiyama.

NATURE AND NURTURE

"This discovery is nice because it shows that the temporal pattern is innate and the aspects that are linked to the morphology of syllables can be influenced by the social environment," says Sébastien Derégnaucourt of University Paris West in France who was not involved in the study. From an evolutionary perspective, Derégnaucourt adds, while species-specific sounds may help birds find mates, learned behavior may help them develop differences between populations that eventually lead to new species.

—Diana Kwon

PROFILE

Singing in the Brain

His first love was dance, but Erich Jarvis has long courted another love—understanding how the brain learns vocalization.

BY ANNA AZVOLINSKY

A single laboratory is a lot to manage, yet Erich Jarvis recently moved to New York from Duke University—where he had been a faculty member in the neurobiology department since 1998—to set up four labs. His primary lab at Rockefeller University, devoted to studying the neurogenetics of language, will continue to attempt to genetically engineer vocal-learning circuits in species that don't possess such a function. It's located in the same building where Jarvis worked as a graduate student and postdoctoral fellow. Also at Rockefeller, he is setting up a vertebrate genomics lab, along with Olivier Fedrigo, to co-lead the vertebrate Genome 10K and the Bird 10,000 Genomes (B10K) Projects. The third Rockefeller-affiliated lab, located at the university's field research center in upstate New York, will house a large transgenic bird colony. The fourth lab, at New York City's Hunter College, will study language function homologies across species, including humans. Jointly with Rockefeller University and Hunter College, Jarvis will also help develop "a program for underrepresented minority students to come and do year-long work in Rockefeller laboratories and in which Rockefeller graduate students and postdocs get experience teaching undergraduates in Hunter courses," he says.

"I'm now considering studying the neurobiology of dance in parrots and humans. If I can do that, I will bring all of my passions together."

Jarvis was trained in molecular biology in Rivka Rudner's lab at Hunter and began his neuroscience career at Rockefeller University in Fernando Nottebohm's group, using songbird communication as a model system to dissect the molecular biology of speech and vocal learning in the brain. "Rockefeller was a place where I had a lot of scientific freedom. The philosophy there is if there is a high probability of an experiment working, then you're not doing the right experiment, and if it has a high probability of failure, then it could make a big impact in science. I am looking forward to that scientific environment, which is hard to find. And I am looking forward to being closer to my family. What I am not looking forward to is the car noise, the pollution, and the cold weather," says the native New Yorker.

Jarvis is also looking forward to opportunities to perform with one of the city's many dance troupes. At the High School of the Performing Arts in NYC, Jarvis majored in ballet because "if you learn something complex, it will make everything easier. I guess I still think that way," says Jarvis. He had scholarships to study at the Joffrey Ballet and the Alvin Ailey schools and still continues to dance and perform. In college, he chose a career in science over one in dance; in graduate school, Jarvis began pioneering work to understand the mechanisms of how songbirds learn to sing, and has since spearheaded evolutionary and sequencing studies of bird species while continuing to research the genetics and neuronal circuitry of vocal communication.

Here, he discusses how *Saturday Night Fever* kick-started his dance career, how his family inspired his scientific pursuits, and why he chose to study the brain.

JARVIS JUMPS IN

Citizen of the world. Jarvis was born in Harlem and grew up in the Bronx and Queens. "Most of my family were singers and musicians and we were expected to become musicians and singers as well. Right before I entered high school, the movie *Saturday Night Fever* came out. I began to imitate John Travolta and was winning dance contests in Connecticut where I lived with my mother, Valeria McCall. I thought maybe I had dance talent, so I auditioned for the High School of the Performing Arts and got in. I realize now how unusual that school was. We were being trained in arts and academics, but on top of that, on how to be a person—to think creatively and to be good citizens and neighbors rather than to compete with one another. That training has influenced who I am now as a scientist. I think that I take a more creative, collaborative, and inclusive approach than some of my colleagues do."

Grasp on life. In high school, Jarvis enjoyed science, and biology in particular. "I was thinking, 'What do I want to do for the rest of my life that would make this world a better place?' because that is what my mother taught me, to do something that has a good impact on society. Of all the things I enjoyed, I felt that as a scientist I had a higher probability of impacting society than as a dancer," he says. Jarvis was also influenced by his father, James Jarvis, who had wanted to become a scientist in the 1960s. "He had a tragic life. He finished high school at 15, but dropped out of college and got into drugs. He was abusive to my mother and to us, his kids, which is why my mother left. I never saw him as a father but as a friend. He was homeless when I was in high school, living in caves in upstate New York to figure out how humans invented civilization. He lived with us for a bit when I lived with my grandfather and he helped me with calculus homework. He had this romantic vision of becoming a scientist that I inherited, but also mental health problems. I learned later that he was partly

COURTESY OF ERICH JARVIS

ERICH JARVIS

Professor, Rockefeller University, New York City
Investigator, Howard Hughes Medical Institute

Greatest Hits

- Showed that brain activation patterns in songbirds differ depending on the social function of the communication
- Discovered relationships between brain pathways for vocal learning in song-learning birds and in humans
- Proposed a theory that vocal-learning circuits in birds and humans likely emerged by duplication of an ancient motor pathway in the brain and that there are no language-specific brain regions separate from speech brain regions
- Co-led an international team that made major revisions in the century-old terminology describing avian brains and contributed to a new understanding of vertebrate brain evolution
- Co-led an international team that sequenced the genomes of 45 bird species and used these plus 3 older sequences to create the first genome-scale phylogenetic tree of a vertebrate group, leading to the discovery that the same specialized form of genes that allow songbirds to learn to sing are also specialized in the human brain regions used to learn speech

schizophrenic and paranoid. He was eventually shot and killed outside one of his caves by a group of teens who were shooting homeless people as gang initiations. All of this was influencing my view of life and my transition from high school to college."

An auspicious start. In 1983, Jarvis entered Hunter College. He fell in love with lab work after joining Rudner's lab, which worked on bacterial protein synthesis genes. Jarvis double majored in biology and mathematics because he couldn't decide whether he wanted "to study how the brain worked or the origins of the universe," he says. In the four years he conducted research in Rudner's lab, Jarvis was an author on seven papers, including three on which he was first author. In one, he mapped the chromosomal organization of the ribosomal RNA genes in *Bacillus subtilis*. Jarvis also convinced Rudner to allow him to give a talk on bacterial genome rearrangements at a genetics conference. "She had said no at first, but I insisted and she told me that if I got the next set of results, I could give the talk," says Jarvis. "For me, a person coming out of a community of color with disadvantages, what was great at Hunter was that the faculty would take you under their wing if you had the ambition and the will, which I don't see at many of the top research schools."

JARVIS'S JOURNEY

A window into learning. For his PhD, Jarvis wanted to apply his molecular biology training to study complex traits such as learning and language. He chose to remain in New York City and attend Rockefeller University. In 1988, he joined Nottebohm's lab, which was studying songbirds because, like humans, these birds had the ability to imitate new sounds. To figure out the molecular mechanisms of vocal learning, Jarvis first used classical conditioning, teaching adult canaries to associate song with a mild shock to their feet. He found that expression of the *ZENK* gene in the forebrain increased in the birds taught the association, suggesting that the gene may be involved in song-linked memory. But Jarvis says he couldn't find any gene changes in the brain region that most interested him, the song-learning nuclei of the forebrain. After finding too many confounding variables in classical conditioning experiments, Jarvis discovered that all he had to do was let the birds sing, and he found singing-driven gene expression in the song-learning nuclei. Since then, he has used the birds' natural behaviors rather than an artificial setup.

Against the odds. Jarvis's first few years at Rockefeller were rough for him. He did not think that he fit into the culture, both

because of the lack of diversity, and because of the sink-or-swim environment. He also had a wife and a young daughter and son to take care of. "But I learned how to hustle, got over the isolation, and then everything picked up," he says. At the end of graduate school, Jarvis ignored career advice to choose a new lab for his postdoctoral training. "This was the best lab in the country, and the world, for what I was doing, and the experiments were finally working, so why would I switch?" he says. Jarvis stayed in Nottebohm's lab for another three years as a postdoc, focusing on the pathways of vocal learning in the brains of songbirds. "This ability to imitate sound is different than regular learning, it's a form of specialized learning not found in many animals," he says. Jarvis found that brain gene expression varies depending on whether it is linked to the production or perception of song in birds, and also that, depending on the type of song a male songbird sings—either a courtship song to a female or one sung alone for practice—the resulting brain activation patterns differed dramatically.

Song to speech. In 1998, Jarvis moved to Duke University to set up his own lab to continue to study song learning as a window into language. "As I was writing grant proposals, I realized the knowledge of how to translate findings in bird brains to human brains was limited. I thought that, at some point, as we make discoveries in songbirds, there would be someone else who would try to test these findings in humans; but I didn't see that happening, so I thought I'd try it theoretically myself," he says. Jarvis began to do meta-analyses to make sense of how songbird research applies to human learning, culling from work going back as far as 150 years. The project culminated in a 2004 paper in which Jarvis hypothesized that vocal learning in both birds and humans evolved from an ancestral and basic neural network within the vertebrate brain. "Researchers were proposing that songbirds are the best model for human speech and showed parallel behaviors, but at the neurobiology level hardly anyone was making that jump between humans and songbirds," he says. In that same time period, Jarvis co-led a consortium of scientists with Tony Reiner of the University of Tennessee Health Science Center that resulted in a major revision of the avian brain nomenclature, which has led to an appreciation of the homologies between the avian and the vertebrate brains. "The result helped justify avian brain experiments for biomedical research because they could now be more easily related to mammal studies."

Jarvis and his students continued to study the evolution of vocal learning, which resulted in the motor theory for the origin of vocal learning, published in 2008. Based on comparisons of neural activity related to vocal learning and movement control in bird brains, Jarvis and his colleagues posited that the neural circuit for song learning in birds, and likely in humans, emerged by duplication of an ancient motor pathway in the brain. "I began to realize that the vocal-learning circuits, including those for human speech that researchers considered cognitively advanced, are really basic motor-learning pathways. It was the first time that I started thinking that this dichotomy in the linguistics and neurobiology communities of a separation between speech and spoken language is false," says Jarvis. "I didn't see any evidence of this separation from the neurobiology studies. No one has found a language part of the brain that is separate from speech production or processing parts."

Bird tree. Since his days at Rockefeller, Jarvis had been thinking about understanding the genes common to songbirds and humans—as well as to other vocal-learning species such as parrots, hummingbirds, bats, whales, and dolphins—but there were few tools to study those genes. By 2010, only two bird genomes had been sequenced, the zebra finch, a songbird, and the chicken, a nonvocal learner, which was not enough to screen for genes associated with vocal learning. It was also unclear if vocal learning really did evolve multiple times among birds. So Jarvis helped lead several consortia, including one at BGI in China that sequenced 45 bird species and resulted in eight articles published in a 2014 special issue of *Science*, along with 20 publications in other journals. "We created the first genome-scale family tree of any vertebrate class, and we confirmed that vocal learning did evolve multiple times," says Jarvis. He and his collaborators are now working on sequencing additional avian genomes.

Comparing the bird genomic data with human brain gene-expression data provided by the Allen Institute for Brain Science in Seattle, "we saw lots of convergent gene expression changes within the vocal-learning brain circuits of birds and in the speech brain regions of humans that were not present in nonhuman primates or in pigeons and doves and mice," he says. "I rarely give up, but before this genomes project, I was thinking, 'I'm not sure I will ever get to finding out whether there are these convergent genes in my lifetime,' because we didn't have the resources. These papers were like a closing of a major chapter for me. Now we can start to manipulate these genes in mice or other species. I don't know if we can do these manipulations and achieve engineering brain circuits for vocal learning behavior, but I am more optimistic now than I was before."

JARVIS'S JUGGLING

Stayin' alive. "At some point, I thought I would give up on dance, but I'm still doing it! I've never stopped dancing, although I did stop performing for a bit. I gave three performances this past year with the James Cobo salsa dance team. Twenty years ago, I thought by the time I was 50 I would be too old for dancing. After I got tenure, I started performing again. I had this fear that I wouldn't accomplish everything I want to before I die, and I see my dancing connected with staying healthy and alive a lot longer to accomplish my scientific goals."

Moving to the beat. In 2008 and 2009, researchers demonstrated that dancing in synch to a rhythm is not just intrinsic to humans, but that other species capable of vocal learning can also spontaneously learn to dance. (See "John Iversen: Brain Beats," page 53.) "In our analysis across species, we found that the genes common to song pathways in different bird species also affect motor circuits involved in coordinating movements in parrots, who are the better vocal learners and dancers," says Jarvis. "This is the start of my going full circle. I'm now considering studying the neurobiology of dance in parrots and humans. If I can do that, I will bring all of my passions together." ■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John Iversen: Brain Beats

Associate Project Scientist, Institute for Neural Computation
University of California, San Diego. Age: 49

BY VIJAY SHANKAR BALAKRISHNAN

When he was a toddler, John Iversen made his first drum set out of pots and pans. But he soon joined his family of percussionists to play real drums and, as a teenager, founded his own rock band. Iversen became curious about the impact of music on humans and animals while studying physics as a Harvard University undergrad.

"You do find many people studying [the] neuroscience of music are musicians, and I'm no different," he says.

Auditory physiologist Nelson Kiang in the Eaton-Peabody Laboratory at Harvard/MIT helped Iversen chart his career path. In 1992, Iversen started his PhD research in the lab of Harvard auditory researcher Christian Brown. But he interacted a lot with Kiang, who studied how the ear transmits auditory signals to the brain and the neural processing that follows. "Most graduate students in science nowadays do research really organized and thought through by their supervisors, but John actually researched [his own idea]," Kiang says. "That was an early indication that he would be a creative scientist, rather than someone who just goes through the motions."

In 2001, Iversen's study of music and the brain took off in earnest after his grad school classmate Ani Patel invited Iversen to join him at the Neurosciences Institute in San Diego to do postdoctoral research in a budding program on the subject.

Working together for about 12 years, the collaborators published several noteworthy papers. One was inspired by a YouTube video that featured a sulphur-crested cockatoo named Snowball rhythmically dancing to musical beats.[1] Patel and Iversen sent slowed-down and sped-up versions of the song from the clip to Snowball's owner, who recorded videos of the bird dancing to those versions and returned them to the researchers, who tested whether the bird's dancing tempo changed.

"John brought a lot of wonderful insight to that project," recalls Patel, who is now at Tufts University. "This brought a lot of interest in the issue of how other animals experience human music . . . and how to understand the evolution of our perception of music," Patel says.

Iversen, now at the University of California, San Diego, studies the neural mechanisms of rhythm perception—trying to pinpoint which brain circuits enable us to dance and detect rhythm in music and speech. He demonstrated the active role of the brain in shaping how a listener perceives a rhythm, a finding that formed the basis of the action simulation for auditory prediction (ASAP) hypothesis.[2,3] According to this hypothesis, listening to rhythms is not solely auditory, but is fundamentally shaped by the brain's motor regions, which predict incoming beats. This suggests that the complex coordination between auditory and motor planning regions involves a neural circuit that was strengthened as humans evolved vocal learning and musical rhythm perception.

With Ramesh Balasubramaniam, a neuroscientist at the University of California, Merced, Iversen is now investigating the ASAP hypothesis in humans by measuring brain function with electroencephalography and directly testing whether auditory perception is disrupted after temporarily disabling motor regions using transcranial magnetic simulation.

"[Iversen] is one of the most original thinkers in the world of music and auditory neuroscience," Balasubramaniam says, "and is one of the few people I know that can bridge a wide gap between evolutionary biology, cognitive psychology, and the brain sciences." ■

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CRISPR Uncut

Early adopters can dive into the CRISPR toolbox with these new proof-of-principle studies.

BY KELLY RAE CHI

By the time you read this article, another boatload of CRISPR tools will have washed up on shore. They will need testing and refinement. Nevertheless, if you ask developers whether you should take these proof-of-principle studies and try them out, you'll only receive encouragement. After all, if it's not yet available from Addgene, it soon will be.

The latest wave of published tools includes new lineage-tracing methods, which can provide a window into the earliest stages of development. New Cas9 inhibitors are now available for the most popular *Streptococcus pyogenes* Cas9 endonucleases, for use in a variety of scenarios to prevent off-target effects. Others strategies merge CRISPR-Cas9 systems with cutting-edge single-cell sequencing technologies. And researchers are, in parallel, moving beyond Cas9 to come up with promising new effector enzymes, such as C2c2, that target RNA for editing and imaging applications, among others.

The Scientist has covered all these advances and more, but here we turn to developers for advice on how to deploy these hot-off-the-bench techniques in your own labs. Here's what they told us.

CRISPR-CAS9 INHIBITION

RESEARCHER: Joseph Bondy-Denomy, Faculty Fellow, University of California, San Francisco

BACKGROUND: Because the Cas9 endonuclease is known to overstay its welcome inside cells, encouraging off-target effects, researchers are working on kill switches for Cas9. "The goal is having a way to turn it off instead of relying on it passively degrading," Bondy-Denomy says. Ideally, Cas9 would find its perfect target, and an inhibitor would then prevent the enzyme from making additional cuts.

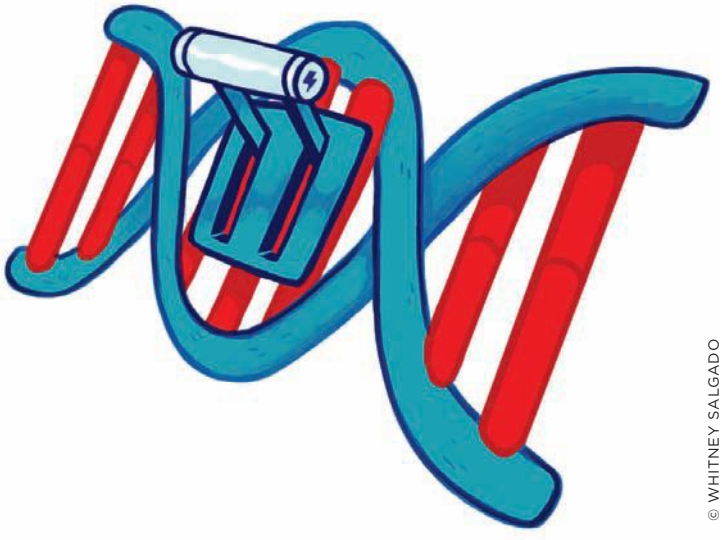
APPROACH: Bondy-Denomy's group came up with four new Cas9 inhibitors, finding that two of them (AcrIIA2 and AcrIIA4) can block actions of the widely used *Streptococcus pyogenes* Cas9, unlike earlier iterations of kill switches that work in other CRISPR-Cas systems (*Cell*, 168:150-58, 2017). This line of work stems from the researchers' observation that, in the battle between bacteria and the viruses that invade them, certain host bacteria, such as strains of the food-borne pathogen *Listeria monocytogenes*, cannot stop viral invasion despite having a CRISPR-Cas9 system with a perfect sequence match to viral DNA tucked into their CRISPR array. Inhibitors are responsible for this phenomenon, and Bondy-Denomy's group continues to search for more.

One possible application is to combine inhibitors with the noncutting CRISPR tool CRISPRi, which is used to dampen expression of specific genes, or CRISPRa, which raises expression (See "Dial It Up, Dial It Down," *The Scientist*, March 2016). Combined, these tools might one day allow users to provide pulses of gene expression or inhibition.

GETTING STARTED: Bondy-Denomy's group plans to make plasmids for all four inhibitors available via Addgene in the near future. AcrIIA2 and AcrIIA4 work in the human cell line HEK293, but keep in mind that it's one cell type and one target. Nevertheless, "anyone can try it with their favorite version of *S. pyogenes* Cas9," he says. "Maybe protein number 1 and 3, which didn't work in our setup, will work for someone else."

Timing—when to apply the inhibitor—will be different in each experimenter's hands, he says. So users will need to check for themselves whether any well-known off-target edits are reduced or eliminated. Of course, check your on-target levels, too. "We would expect some on-target activity to go away, but we don't know yet," he says.

FUTURE: The group is testing the inhibitors using guide RNAs—the stretches of sequence that allow Cas9 to target a specific location in the genome—that are known to be problematic for introducing off-target mutations. This is important because



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researchers are often hamstrung, Bondy-Denomy says: they want to fix a point mutation at a specific place, and that target needs to be followed by a protospacer-adjacent motif (PAM), a specific 2–6 base-pair sequence (immediately following the target DNA) that is required for Cas9 to bind to the target DNA. The desired edit should change the PAM sequence, or you risk cutting the target site again and destroying your work. So, the inhibitors provide a mechanism for Cas9 to be active transiently.

PERTURB-SEQ

RESEARCHERS: Jonathan Weissman, Professor, Department of Cellular Molecular Pharmacology, University of California, San Francisco, and Aviv Regev, Core Member, Broad Institute of MIT and Harvard

BACKGROUND: Along with an expanding CRISPR-Cas9 toolbox, single-cell RNA sequencing has advanced rapidly in the past few years. Academic researchers have come up with droplet-based methods (See "Scaling to Singles," *The Scientist*, May 2016), and the first commercial platforms are coming online.

APPROACH: A trio of studies published in *Cell* show that it's possible to deploy the CRISPR-Cas9 toolbox—in this case both for gene editing and gene suppression—and conduct high-throughput single-cell RNA-seq (*Cell*, 167:1853-66, 2016; *Cell*, 167:1867-82, 2016; *Cell*, 167:1883-96, 2016).

Weissman's and Regev's new technique is called Perturb-seq, and the key to making it work was being able to encode the CRISPR-based perturbations in the transcriptomes of single cells. Most high-throughput single-cell RNA-seq methods rely on the ability to capture the 3' polyadenylated tail ends of RNA, but guide RNAs are generally not equipped with polyA tails and can't be captured directly. So, postdoctoral researchers Thomas Norman and Britt Adamson in Weissman's group came up with a barcoding strategy to identify the guides. "Here, we can a priori tag cells that will be treated differently, and later map those tags back to specific perturbations," Adamson says. Another crucial step was to develop an analytical pipeline to parse the massive amounts of data. (See "Massively Parallel Perturbations" on page 25.)

GETTING STARTED: The scientists used 10X Genomics's platform for single-cell RNA-seq (Chromium Single Cell 3' Solution), which is so new that many may not have access to it yet. But Perturb-seq also works in 96-well plates and using the Drop-seq method, Norman says. What's more, you should be able to use your favorite Cas9 variant, he adds.

The vectors they used to barcode and express guide RNAs will soon be available via Addgene. Using this method, researchers can (in theory) pool cells from separate experiments in order to cut costs. Data analysis can be done on a standard computing cluster; but note that it will likely take several months (at least) to mine the data, Norman says.

FUTURE: Weissman's group plans to release a more-detailed protocol, and he and Regev's group will release some of the computational tools they have developed for these papers. "We would like to make this as accessible a technology as possible," Adamson says.

To that end, Weissman's group is also working on improved library preparation protocols, such as narrowing the focus to a more limited set of genes in order to cut experimental costs. In parallel, they are also hoping to address biological and logistical obstacles to scaling up sequencing to the entire coding genome.

HOMING GUIDE RNAS

RESEARCHERS: Prashant Mali, Assistant Professor, Department of Bioengineering, University of California, San Diego, and George Church, Professor, Department of Genetics, Harvard Medical School

BACKGROUND: Lineage tracing has been crucial to developmental biology (see "How to Track Cell Lineages As They Develop," *The Scientist*, December 2016). In the past year, several groups have deployed CRISPR-Cas9 approaches to track cell fate. All of

them work by tracking Cas9's edits in the genome as they accumulate during development from a single cell.

APPROACH: The newest spin is a homing guide RNA that directs Cas9 to its own DNA locus as its target, thus cutting itself—effectively serving as a diversifying barcode that Mali's and Church's groups can track in cultured cell populations. The approach is unique in that the edited homing guide RNAs retain the ability to target themselves, so the lifetime of these guides is potentially longer than the barcodes used in other lineage tracing approaches. In addition, because the barcodes are transcribed, researchers can potentially read them out using RNA detection approaches like FISSEQ (*F*luorescent *I*n *S*itu *SEQ*uencing), a method developed in the Church lab (*Nature Protocols*, 10:442-58, 2015). Mali points out, however, that the new study amplified and detected the RNAs rather than sequencing them (*Nature Methods*, 14:195-200, 2017).

GETTING STARTED: "Fundamentally, the tool is really simple to implement. It boils down to creating cells which have particular barcodes present in them," Mali says. Detection doesn't have to be by FISSEQ, but any single-cell sequencing method could potentially work.

Just like standard guide RNAs, homing guide RNAs need to be experimentally tested. Some of the self-mutations formed upon cutting trigger large insertions or deletions that eliminate their homing activity. Others can stay active for a long period of time. "Trying to find the right mix and match for your particular application is going to be critical," Mali says. For example, if you want to be able to barcode across multiple cell divisions, then you need a homing guide that will stick around for a longer period of time. "At least as of this moment, we don't have precise rules for how to choose the best homing guide RNAs," he adds.

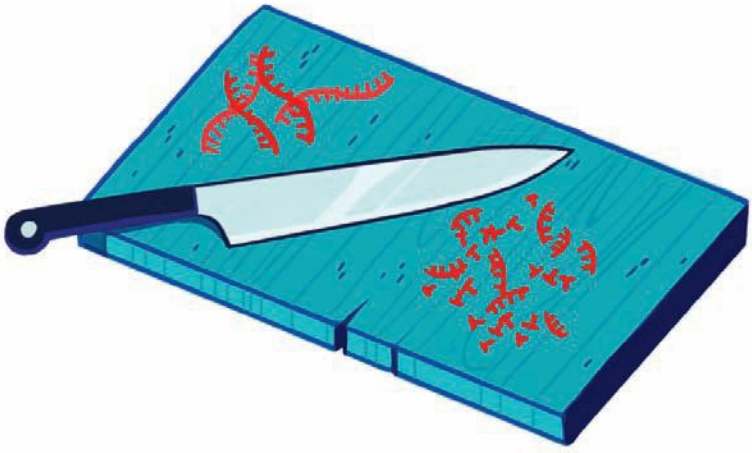
FUTURE: Theoretically, the barcodes can generate a sufficient number of variants to cover all of the various cell types in an entire mouse brain. The group's current focus is on taking their studies in vivo.

CUTTING RNA

RESEARCHERS: Feng Zhang, Core Member, Broad Institute of MIT and Harvard, and Eugene Koonin, Senior Investigator, National Institutes of Health

BACKGROUND: Because it's a genome-editing tool, conventional CRISPR-Cas9 is less than ideal for studying the plethora of genes that are transcribed but never translated—that is, the noncoding genome. Being able to manipulate RNA directly allows researchers to better understand RNA and to develop RNA-based therapeutics such as antiviral drugs.

APPROACH: In a proof-of-concept study, Zhang's and Koonin's groups collaborated to characterize the new CRISPR effector



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C2c2, which cuts specific targets in single-stranded RNA (*Science,* 353:aaf5573, 2016) and showed that it can degrade a specific mRNA in *E. coli*. (See "RNA-Targeting CRISPR," *The Scientist,* June 2, 2016). The two teams have found many additional "flavors" of C2c2. With various collaborators, they are working with about 16 of these orthologs, and testing them for different applications, such tracking RNA in living cells.

Unlike RNA interference tools for quelling gene expression, C2c2 can be localized within the cell. "You can take it to the nucleus and have it knock down there. It becomes a powerful tool for probing the [expressed] noncoding genome," says Omar Abudayyeh, a graduate student in Zhang's group.

GETTING STARTED: The original C2c2 plasmid (from the bacterium *Leptotrichia shahii*) is available from Addgene, but note that it is not optimized for use in mammalian cells. It's more likely to work in bacteria, or in nonmammalian eukaryotes such as plants, though they haven't been tried yet, Abudayyeh says.

Even with the current ortholog, there is guide-to-guide variability when trying to target a transcript, Abudayyeh says. That's because of not only the sequence itself but also the accessibility of the transcript. Many transcripts have secondary structures or are bound by proteins that obscure the sequence. To get around this problem, the group suggests tiling your target with many evenly spaced guides. In addition, computational tools predicting RNA structure may help inform how successful you'll be with a particular target.

Of course, it's important to check for protein expression to see whether your C2c2 works. In addition, they often tag C2c2 with an antibody to check its expression, says Jonathan Gootenberg, a graduate student in Zhang's and Regev's labs at Broad.

FUTURE: Besides characterizing new orthologs of C2c2, the Broad groups are conducting guide-tiling arrays, which are like microarrays except that the oligonucleotide probes are guides, in order to develop a set of rules that can then help people predict success with guides. ■

China's Ethical Inflection Point

Several initiatives aim to improve research integrity in the country, but recent high-profile cases of misconduct highlight a lingering problem.

BY BEN ANDREW HENRY

In 1999, China was responsible for 3.5 percent of scientific studies published globally, according to the journal-ranking database SCImago. By 2015, that number had leapt to 18 percent. Scientific output has exploded in the country alongside its flourishing economy and technological acceleration, leaving it second only to the U.S. in national research spending.

Growth, however, has not come without growing pains. In the late 1990s, three high-profile cases of plagiarism by Chinese researchers set into motion a national discussion over research integrity that continues today. Chinese academics warned at the time that if the country were to realize its potential as a research powerhouse, its institutions needed to crack down on dishonest research practices—not just plagiarism, but financial conflicts of interest and outright falsification.

Over the next two decades, Chinese government and academic institutions established ethics policies and educated students in how to avoid misconduct. But a string of high-profile retractions in 2015 raised doubts about the success of those efforts. In one widely publicized spate of retractions, BioMed Central pulled 43 papers for falsified peer review; 41 were written by researchers from China.

Scientific misconduct remains a thorn embedded in the side of China's research enterprise, as a 2015 report from Nature Publishing Group observed. The country's reputation for misconduct may well be harsher than is fair, given that misconduct is a problem found virtually everywhere. But the authors of the report write that the burden of this reputation "makes the need to tackle misconduct all the more important."

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The roots

By the end of the 1990s, national and international media had started to take notice of troubling research integrity breaches within China's academic community. Over the course of a few years, researchers at three Chinese universities were exposed for lifting passages from other scientists' publications, in several instances passing off entire manuscripts as their own. Although punishments were dealt, a 1996 commentary in *Science* noted that China's scientific institutions lacked clear plans for preventing future misconduct, largely because none of the leading funders or regulatory bodies had gathered any information on the extent of the problem.

In the decades prior to these revelations, Chinese society had undergone sweeping and at times turbulent changes. After the end of the Cultural Revolution in 1976, the Chinese economy remodeled itself from agrarian to industrial, and the government took a keen interest in advancing scientific research. Cities modernized and their populations ballooned, and government grants poured funding into academia. But, the increase in research was not accompanied by parallel growth in regulatory oversight to prevent misconduct, says bioethicist David Resnik, coauthor of a 2010 historical assessment of research integrity in China (*Bioethics*, 10:164-71). The result was a research cul-

ture that pressured scientists to succeed and permitted ethical corner-cutting, such as plagiarism, in service of that success.

Cultural norms likely exacerbated the situation. In any country, the power and authority allocated to PIs can discourage students, postdocs, and other lab members from speaking up about research practices that violate ethical codes. In China, the cultural value placed on respecting authority figures makes this power dynamic even harder to surmount, Resnik observes. The language barrier also contributed to misconduct, as some scientists in China viewed borrowing phrases or sentences in English from a published text as an acceptable way to learn the language, a 2007 study of Chinese PhD students suggested (*Applied Linguistics*, 28:440-65).

Our country has been harsh in wording but weak in action in handling academic misconduct.

—Pseudonymous writer Muding Bai in the Chinese newspaper *People's Daily* (translated)

One concerning practice in Chinese universities—in the early 2000s and, by some accounts, continuing today—was the use of monetary incentives for academics to publish in high-impact, international journals. According to a 2008 report in *Science*, the typical baseline salary for a PI in China at the time equated to no more than a few thousand US dollars per year, but publishing a paper in *Science* or *Nature* might earn bonuses upwards of $2,500. Such financial rewards only magnify the "publish or perish" mentality frequently blamed for incentivizing researchers around the world to falsify work.

The simmering problem of research integrity in China eventually came to a boil, in part due to the efforts of Shi-min Fang, a Chinese biochemist and bioinformatics consultant based in San Diego. Fang had grown frustrated with the prevalence of shoddy research in China and with the unwillingness of researchers to challenge their peers. In 2000, he took matters into his own hands, turning his website New Threads into a forum for criticizing studies that made questionable claims and researchers with compromising financial ties. Scientists could anonymously post criticism as well, and the site soon teemed with accusations of research misconduct that might otherwise never have surfaced.

Allegations made on Fang's site toppled some of country's most visible scientists, sparking controversy along with praise. The computer scientist Jin Chen, for example, was at one point held up as a national hero for designing high-speed microchips, China's answer to equivalent chips made in the West. But in 2006, a New Threads post alleging the chips were a fraud triggered a government investigation that wrested Chen from glory: the chips, the investigation revealed, were simply Motorola chips with the brand scratched off and replaced by Chen's own brand.

Meanwhile, a misconduct scandal elsewhere in Asia served as another warning sign to Chinese academics about the urgency of addressing research integrity. The same year that Chen was called out on New Threads, *Science* retracted two papers by the lauded South Korean stem cell biologist Woo Suk Hwang, on the grounds that he had falsified data. "When that scandal came, it made a lot of us think hard," says Danny Chan, a stem cell biologist at the University of Hong Kong. "Most of us were aware about the issue of responsible research conduct," but the Hwang case made it clear that integrity needed to take high priority, he says.

Turning a corner?

In 2007, the China Association for Science and Technology (CAST), a regulatory agency connecting the Chinese government with its academic institutions, partnered with the American Association for the Advancement of Science (AAAS) to host a conference on research misconduct. Representatives from both organizations talked over the problem and possible solutions for two days in Beijing. "We want to send a message to the whole world that it is our purpose to promote the development of ethics and scientific integrity," a CAST official said in a statement about the event.

One way the Chinese government hopes to improve the country's research integrity is simply by educating researchers on what's allowed and what's not. In 2015, for example, CAST issued a list entitled "The Five Don'ts of Academic Publishing" that emphasizes in detail that "paper brokers"—companies whose services range from above-board assistance with English-language editing to malfeasant practices such as fabrication and peer review manipulation—should have no role in scientific publishing greater than fixing run-on sentences. And in 2011, Chinese leaders instituted a campaign to deliver lectures on research ethics to graduate students. In less than four years, 4.4 million graduate students sat in on Ministry of Education–sponsored lectures on research ethics, according to AAAS.

Another promising step toward implementing ethical research practices in China was the 2013 appointment of Wei Yang to lead the National Natural Sciences Foundation (NSFC), a major regulatory and funding agency. Yang, a former president of Zhejiang University, built himself a reputation for cracking down on scientific misconduct. When a plagiarism scandal blew through his university in 2008, Yang fired the researcher involved and punished others connected to the case—the first of around 40 misconduct cases Yang would address over the next two years with equal severity, according to a 2012 feature in *Nature*.

The various efforts from Chinese institutions to clean up misconduct are more than lip service in response to pressure from the West, says Resnik. "[Chinese researchers] have a lot at stake in this. . . . They don't want a reputation for doing unethical or biased research."

Persistent problems

Evidence for the success of China's efforts remains ambiguous, however, in part because independent data on research conduct in the country are scarce. And a handful of surveys suggest that the prob-

lem lingers. A survey in 2010 and 2011 of 1,800 academic faculty members at the University of Hong Kong found that only 12 percent to 20 percent believed fabrication of research was "harmful and deserving of punishment." And a 2012 survey of 30,000 Chinese researchers found that around half admitted to ethical violations such as improper attribution of sources and submitting manuscripts to multiple journals, according to AAAS. (For comparison, a 2009 meta-analysis of surveys in the U.S. found that roughly one-third of scientists admitted to any form of research misconduct.) Plagiarism in particular appears to still be prevalent in publications from Chinese researchers. A 2014 study found that while China trailed the United States in its total number of retractions, the proportion of those retractions due to plagiarism was twice as high as the US figure.

The recent wave of retractions over faked peer review has brought renewed, unwanted attention to China's research integrity. Since 2012, a total of 250 studies have been retracted on the grounds that authors provided false email addresses for suggested reviewers or otherwise tampered with the peer review process, and a recent assessment of the retracted studies found 75 percent were written by Chinese authors. A CAST investigation into the incident directed blame toward the country's well-established network of paper brokers. "This third-party industry offering 'scientific services' does not have a self-regulatory organization and is not subject to penalty," the statement read.

Others have blamed China's research institutions for failing to adequately enforce misconduct policies. An op-ed published last November in the Chinese newspaper *People's Daily* called on universities to dole out stiffer, more-consistent punishment for misconduct and to pursue investigations that could identify cases of wrongdoing before invalid research is published. "Our country has been harsh in wording but weak in action in handling academic misconduct," wrote the author, under the pseudonym Muding Bai [translation courtesy of Ping Sun, a former official within China's Ministry of Science and Technology; edited for grammar and clarity]. "They often turn a blind eye to academic misconduct and perform no investigation into cases unless allegations are made. As a result, 'zero tolerance' has amounted to 'zero action.'"

Despite such lingering problems, many are optimistic about the future of research integrity—and scientific discovery—in China. Yang observed in a 2016 perspective in *Nature* that NSFC funding has ticked steadily upward while allegations of misconduct around grant proposals submitted to the agency have fallen. He writes: "China is rising rapidly up the global scientific ranks by every measure." ■

Caterwauling for Science

Tone deafness and a love of music made me the perfect research subject for scientists who study congenital amusia.

BY TIM FALCONER

I've spent my career bothering people. As a journalist and author, I hang around and watch what folks do, and I ask too many questions, some better than others. Later, I have follow-up queries and clarification requests, and I bug them for those stats they promised to provide me. But something different happened when I started researching congenital amusia, the scientific term for tone deafness present at birth, for my new book, *Bad Singer*. The scientists were as interested in me as I was in them.

My idea was to learn to sing and then write about the experience as a way to explore the science of singing. After my second voice lesson, I went to the Université de Montréal's International Laboratory for Brain, Music, and Sound Research (BRAMS). I fully expected Isabelle Peretz, a pioneer in amusia research, to say I was just untrained. Instead, she diagnosed me as amusic.

"So this means what?" I asked.

"We would love to test you more."

The BRAMS researchers weren't alone. While still at Harvard's Music and Neuroimaging Lab, Psyche Loui—who now leads Wesleyan University's Music, Imaging, and Neural Dynamics (MIND) Lab—identified a neural pathway called the arcuate fasciculus as the culprit of congenital amusia. So I emailed her to set up an interview. She said sure—and asked if I'd be willing to undergo an fMRI scan.

And I'd barely started telling my story to Frank Russo, who runs Ryerson University's Science of Music, Auditory Research, and Technology (SMART) Lab in Toronto, before he blurted out, "Sorry, I'm restraining myself from wanting to sign you up for all kinds of research and figuring what we can do with you."

I'm an unusual amusic because I love music—many fellow sufferers are indifferent or actively dislike it—and I wanted to learn to sing. Since I hadn't had a lesson in six months, Russo suggested I restart my training while his lab tracked my progress.

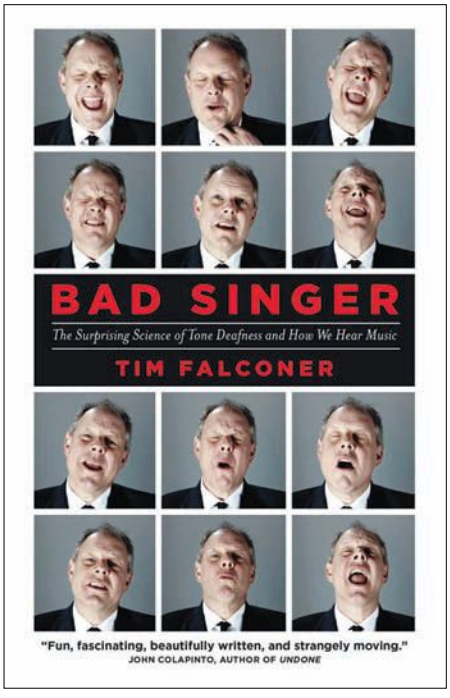
I'd listen to two tones and then sing them and say if the second was higher or lower than the first. I also had to deliver several renditions of "Happy Birthday," a song I soon learned to hate.

After seven months, my ability to match pitch hadn't improved (though in my lessons, I typically matched my vocal coach's notes, because it's easier to copy a human voice than an instrument). But something weird was going on: if, say, the second note was higher, I'd often sing the two sequential notes in the right order, but then confidently say the second was lower. This supported the theory that amusics actually can hear pitch, but they don't have conscious access to that information.

As for "Happy Birthday," I wasn't hitting all the notes, but my pitch improved slightly, and my rhythm was much better. More important than these data points, because so much of music perception evades measurement, Russo invited 14 musicians to listen to and score randomized versions of me singing "Happy Birthday," and they could hear my progress.

Later, Russo and Peretz combined their research to write about me for *Neurocase*, a journal devoted to neuroscience case studies (22:526-37, 2016). Reading "Effects of vocal training in a musicophile with congenital amusia" was an odd experience. Although I've read about myself before (book reviews, interviews, and so on), this was all so . . . clinical.

Peretz and Russo had shared most of the results, at least in a general sense, when I was writing the book, but this was more detailed. And some of the information was new to me. For example, I was fascinated that a brief personality assessment questionnaire—which I don't remember taking—fairly accurately predicted my musical tastes (indie rock, alt-country, country, blues, R&B, and reggae).



House of Anansi Press, May 2016

Still, the conclusion that the limited improvements in my singing were "transient" was disappointing, even though I was already painfully aware that was true.

So, with no singing career in my future, I'll stick with my day job and continue to ask questions for a living. But it was fun being studied for a change, and at least the researchers had called me "a remarkable case." ■

Tim Falconer is an award-winning journalist and author of four books of nonfiction. He mentors creative-nonfiction writers in the MFA program at the University of King's College in Halifax, Nova Scotia, and is an editor in the literary journalism program at Alberta's Banff Centre for Arts and Creativity. Read an excerpt from Bad Singer: The Surprising Science of Tone Deafness and How We Hear Music *at the-scientist.com.*

COMING**SOON** | Single-Cell Phenotypic Analysis: Breaking the Law of Averages

Population-scale cellular analysis has provided many valuable insights into general trends of cell behavior and expression, but rare and low-level events can get lost in the lysate. Averaging the signals from an entire culture can drown out the least prevalent but most important phenotypes. That's why single-cell analysis has made such an impact, enabling higher throughputs, rare-event detection, and more-precise and meaningful analyses. To explore this exciting new frontier, *The Scientist* is bringing together a panel of experts to share their experience with single-cell phenotypic analysis and to reflect on the clarity it provides. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

ANDREW THORBURN, DPhil
Professor and Chair, Department of Pharmacology
University of Colorado, Anschutz Medical Campus

RYAN BRINKMAN, PhD
Professor, Medical Genetics
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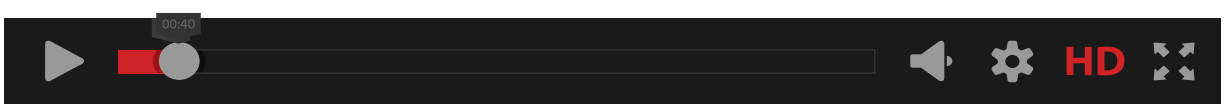

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SALVADOR SIERRA-SAN NICOLÁS, MD, PhD
Postdoctoral Fellow, Department of Pharmacological Sciences
Icahn School of Medicine at Mount Sinai

SOPHIA SCHEDIN-WEISS, PhD
Associate Professor, Center for Alzheimer Research
Department of Neurobiology, Care Sciences and Society
Karolinska Institute

TOPICS COVERED:

- Using proximity-ligation assays to tease out protein interactions in neurodegenerative disease
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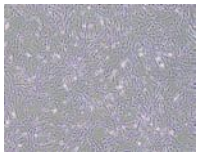
Lonza Launches IPF Primary Cells



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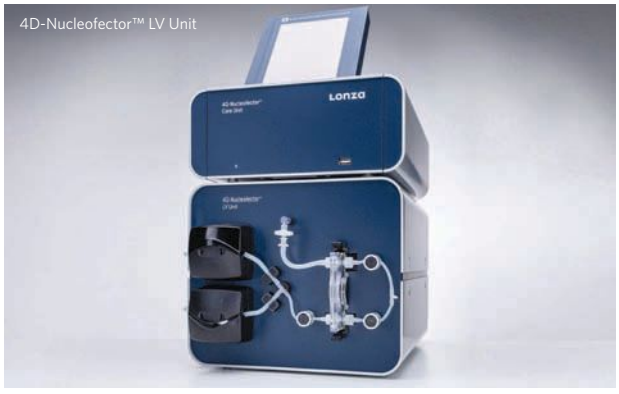

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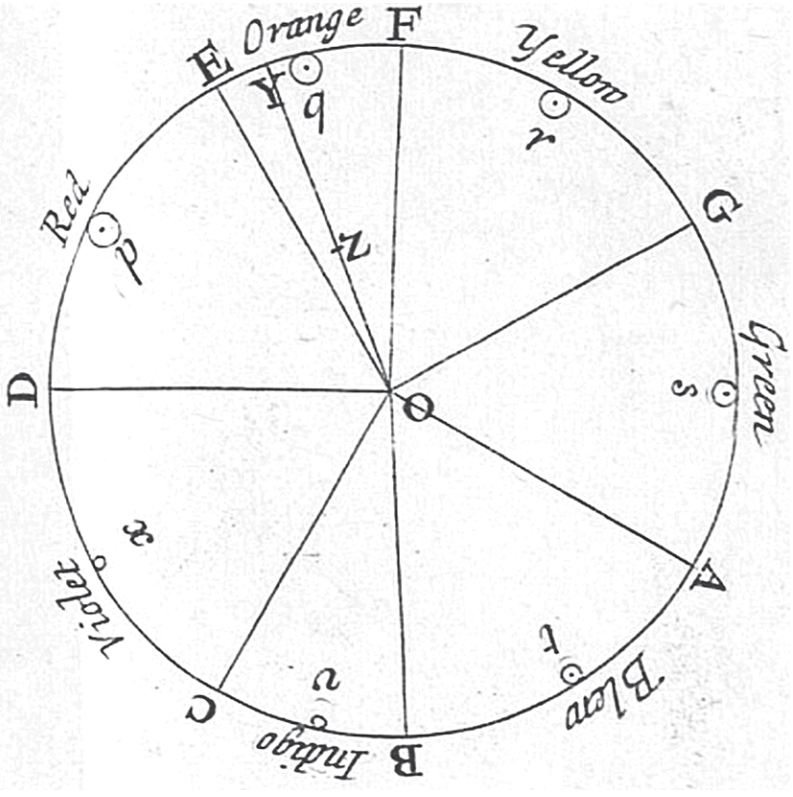
Newton's Color Theory, ca. 1665

BY ASHLEY P. TAYLOR



COLOR NOTES: In Newton's color wheel, in which the colors are arranged clockwise in the order they appear in the rainbow, each "spoke" of the wheel is assigned a letter. These letters correspond to the notes of the musical scale (in this case—the Dorian mode—the scale starts on D with no sharps or flats). Newton devised this color-music analogy because he thought that the color violet was a kind of recurrence of the color red in the same way that musical notes recur octaves apart. He introduced orange and indigo at the points in the scale where half steps occur: between E and F (orange) and B and C (indigo) to complete the octave.

ISAAK NEWTON, WIKMEDIA COMMONS

Around 1665, when Isaac Newton first passed white light through a prism and watched it fan out into a rainbow, he identified seven constituent colors—red, orange, yellow, green, blue, indigo, and violet—not necessarily because that's how many hues he saw, but because he thought that the colors of the rainbow were analogous to the notes of the musical scale.

Naming seven colors to correspond to seven notes is "a kind of very strange and interesting thing for him to have done," says Peter Pesic, physicist, pianist, and author of the 2014 book *Music and the Making of Modern Science*. "It has no justification in experiment exactly; it just represents something that he's imposing upon the color spectrum by analogy with music."

Of his rainbow experiment Newton wrote that he had projected white light through a prism onto a wall and had a friend mark the boundaries between the colors, which Newton then named. In his diagrams, which showed how colors corresponded to notes, Newton introduced two colors—orange and indigo—corresponding to half steps in the octatonic scale. Whether Newton's friend delineated indigo and orange on the wall or whether Newton added those colors to his diagrams in order to better fit his analogy is unclear, Pesic says. In any case, Newton's inclusion of those two colors had lasting consequences, Pesic wrote in his book: "For those who came after, Newton's musical analogy is the source of the widely held opinion that orange and indigo are actually intrinsic in the spectrum, despite the great difficulty (if not impossibility) of distinguishing indigo from blue, or orange from yellow, in spectra."

Newton persisted with his color theory despite later data he had collected suggesting it was incorrect. When studying what are now called Newton's rings—as seen, for example, in the rainbow of color in oily puddles—he noted that, according to the relationship between radii of colored rings, the range from red to violet was equivalent not to an octave but to something more like a major sixth. According to Pesic, rather than changing his theory to match the data, Newton came up with an erroneous explanation of how a major sixth was equivalent to an octave.

But as both musicians and physicists know, the two are not equivalent. In physics terminology, an octave is the frequency range from x to 2x, and that premise holds true for musical octaves. If light behaved like music, then photon frequencies of the spectrum would also range from x to 2x, and their wavelengths, inversely proportional to their frequencies, would too. Instead, the wavelengths of visible light range from 400 to 700 nanometers, which, if translated to sound waves, would be approximately equivalent to a major sixth, Pesic says.

Although Newton's color-music analogy falls apart, his prism experiments showed that white light is actually a mix of different-colored lights, and this work was "a crucial step toward understanding the nature of light more deeply," Pesic says. And even if you can't make out indigo in the rainbow, you probably know ROY G BIV, which Pesic calls "a conventional expression of (and homage to) Newton's choice [to name seven colors in analogy to music]—even though almost everyone has forgotten or did not know the odd story of its origin." ■

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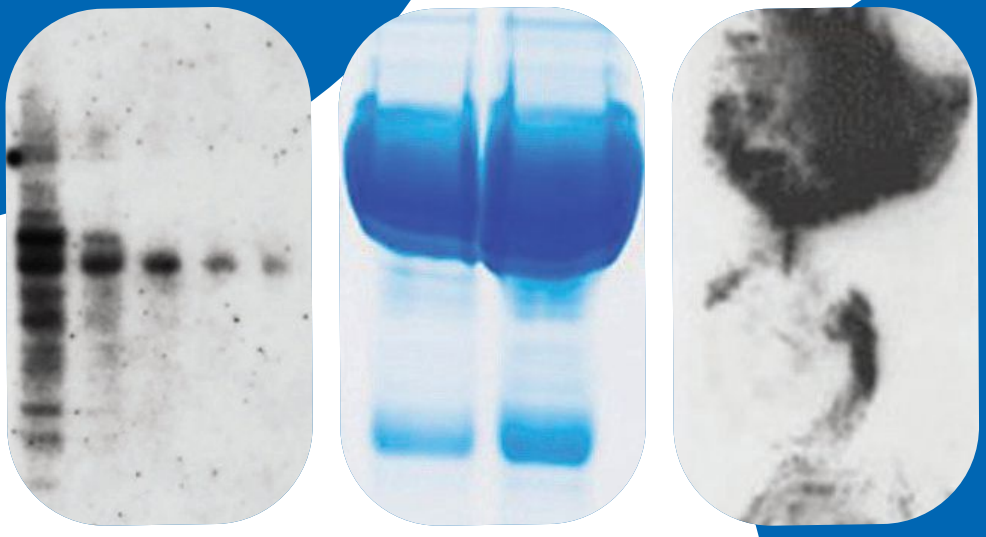

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