NEWS FEATURE

Singing in the brain

Songbirds are helping scientists decipher the foundations of human speech. But new work on bats may provide missing pieces of the puzzle.

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Several times a week, neuroscientists at the University of California, Berkeley, shuttle a furry brown bat named Cooper down the hall to visit a computer that offers sips of fresh smoothie in exchange for conversation. Once inside a customized sound chamber, Cooper lets out a high-pitched trill and cranes his neck eagerly toward a nozzle that dispenses his sweet treat.

For the past year, Michael Yartsev's team has been training Cooper and a handful of other bats to "chat" with the computer. It's the first step in a project aimed at cracking the mysteries of how human speech works, a component of which is vocal learning, or the ability to imitate and create new sounds. Humans share this ability with a select group of animals, including whales, seals, dolphins, elephants, parrots, hummingbirds, and songbirds (1). Increasing evidence suggests that some bats are vocal learners too (2).

That's welcome news for researchers who use songbirds as the animal model of choice to understand vocal learning. These birds are revealing much about the neurobiology and genetics of vocal learning. Even so, 600 million years of evolution and radically different brain architectures separate birds from humans. As a new mammalian model, the bat could bring researchers even closer to the answers they seek. Many hope that bats like Cooper will help bridge the evolutionary gap between birds and humans.

"We have a lot of catching up to do to the bird work, but there's also the excitement that comes with being a really young field," says Sonja Vernes, a

In recent years, a handful of vocal learning researchers have turned their attention to bats' social vocalizations, including the Egyptian fruit bat's repertoire of screeches, trills, and other cries. Image courtesy of © Steve Gettle/Minden Pictures.

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neurogeneticist at the Max Planck Institute for Psycholinguistics in Nijmegen, The Netherlands.

Bird Brains

Vocal learning, in general, refers to the production of novel sounds by learning or imitation. But what counts as "novel" is debatable. Some scientists say that it includes the ability to modify innate sounds in response to social experience. Others limit the definition to the creation of entirely new sounds, as when a baby speaks its first words.

By this measure, birds provide an especially clear and dramatic example of vocal learning. Around the 1950s, scientists began observing striking parallels between avian and human vocal learning. Birds don't learn to sing properly—and babies don't learn to speak normally—without early exposure to adult vocalizations, a requirement not shared by most other animals. Young male songbirds listen to and form a memory of an adult tutor's song (usually the father's), then gradually shape their immature "babbling" sounds to match it through trial-and-error practice, similar to how babies learn (3). In recent years, modern molecular and electrophysiological techniques have provided growing evidence that these behavioral similarities reflect some common biology.

This is surprising, because a bird's brain looks quite different from a mammal's. In particular, birds lack the six-layered cerebral cortex that encases mammalian brains. Especially enlarged in humans, the cortex is associated with "higher" functions, such as learning and cognition. The absence of a layered cortex led to the early belief that the avian brain consisted entirely of the more "primitive" basal ganglia, a collection of subcortical brain areas involved in motor planning and coordination. Birds also lacked the canonical cortical-basal ganglia circuits believed to facilitate complex, learned movements in mammals. "This made people think birds couldn't learn anything—that they were just dumb and automatic, acting purely on instinct," recalls neuroscientist Sarah M. N. Woolley at Columbia University in New York.

But by the early 2000s, a different picture began to emerge, helped by new techniques for tracing connections between neurons and labeling different cell types and signaling molecules. Researchers showed that in songbirds, neurons dedicated to song are organized in clusters—called nuclei—that are roughly analogous to different layers of the mammalian cortex. Not every songbird nucleus has been precisely matched to a layer of the mammalian cortex, but some nuclei share key features with specific layers. In 2004, an international group of researchers, called the Avian Brain Nomenclature Consortium, renamed many parts of the bird brain to highlight these proposed analogies (4).

Since then, additional work has confirmed and deepened many of these comparisons. Just as each mammalian cortical layer has a unique pattern of gene expression, researchers have found similar patterns in nuclei involved in birdsong. The findings suggest that certain cell types are shared across the two systems, but are configured into clumps in birds and layers in

A juvenile zebra finch learns song from his adult tutor. Image courtesy of Sarah M. N. Woolley.

mammals (5). "The macrostructure throws you off, but if you look at the cell types that are there and which types are connected to which other types… they're very similar," says Woolley.

For example, in the avian brain region most similar to the mammalian auditory cortex, connections between certain groups of avian neurons resemble the wiring patterns between the analogous brain regions in mammals (6). In both birds and mammals, these groups of neurons show similar firing patterns (with some firing more rapidly and others less so), and respond in the same order, suggesting that information is processed in much the same manner (7).

Of Genes and Songs

Given these similarities, Woolley and other avian neuroscientists see potential for uncovering the circuits and computations that give rise to vocal learning in humans. But others take a more conservative view. Evolutionary and cognitive biologist Tecumseh Fitch at the University of Vienna in Austria argues that in some cases, anatomical differences between the bird and human brain are too great for the two organs to be properly compared. Instead, he believes that songbirds may be more useful for studying the genes involved in vocal learning. "Once we get to the genetic level, we have a whole other foundation we share with birds," says Fitch. "Many of the same genes are playing the same roles. It's what some people are calling a 'deep homology.'"

One such gene is *FOXP2*, the first gene discovered to cause a language disorder in humans. In 2001, researchers reported that about half the members of a British family carry a *FOXP2* mutation that results in impaired grammar and language comprehension. Affected family members also struggle to coordinate sequences of oral and facial movements to produce speech (8). *FOXP2* encodes a protein that regulates the expression of a multitude of other genes, and scientists are still figuring out how the whole system influences vocal communication.

Genetic similarities with birds are providing clues. In the zebra finch (*Taeniopygia guttata*), the most widely studied songbird, *FoxP2* is expressed in similar patterns as in the human brain, showing up in the thalamus (an integration and relay station for sensory information), the cerebellum (involved in fine motor coordination), and at especially high levels in the basal ganglia. As in humans, disruptions to *FoxP2* activity can have pronounced effects on vocal learning in songbirds.

At the Free University of Berlin, neuroscientists led by Constance Scharff have found that suppressing FoxP2 protein levels in a key basal ganglia structure during song learning prevents young zebra finches from imitating their tutors properly. The birds go on to produce abnormally variable songs, dropping some syllables and performing others inaccurately (9). But artificially boosting FoxP2 levels in the same brain region also interferes with song learning, as Stephanie White's team has discovered at the University of California, Los Angeles (10). Together, these results suggest that precisely regulated FoxP2 levels may be critical to vocal learning, says White.

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—Sonja Vernes

Other genes could have important roles as well. In a 2014 study comparing vocal learning and nonlearning species, led by neuroscientist Erich Jarvis, researchers identified roughly 50 genes with potential links to this special skill (11). These genes showed similar expression patterns in songbird and human brains, patterns not found in vocal nonlearning birds, such as doves, and nonhuman primates, such as macaques. In particular, the genes showed either increased or decreased expression in brain areas that control the vocal organ (the syrinx in birds and the larynx in humans) compared to other, neighboring brain areas.

Some of these genes are known to be regulated by *FoxP2*, including *SLIT1*, which is involved in guiding neurons to make new connections. Jarvis suspects that *SLIT1* could be a major player in vocal learning, as *SLIT* genes are involved in biological pathways that are disrupted in some forms of autism, dyslexia, and speech and language disorders (12). "Now you can take these genes and study them in the bird," says Jarvis, a professor at the Rockefeller University in New York. "You couldn't do that with another animal model."

From the Shadows

That won't be the case for long, if bat researchers have their say. "I really want to have another model system that's in between birds and humans," says Vernes. With bats, "you can get closer to the questions you want to ask in humans."

While pocket-sized bats have been used for years to study echolocation and spatial navigation, their vocal learning skills remain relatively unstudied. Examination of some 50 bat species has uncovered four so far with varying degrees of vocal learning. Some bats learn to match their calls to the acoustic signatures of those around them; others pass down territorial songs from father to son. But the vocal skills of most of the roughly 1,300 bat species are unknown, and evolutionary biologist Mirjam Knörnschild at the Free University of Berlin suspects that many more vocal learners will soon be discovered.

Every year for the past decade, Knörnschild has journeyed to the jungles of Central America to study the greater sac-winged bat (*Saccopteryx bilineata*), one of the clearest examples of vocal learning among bats. Males pick up territorial songs from their fathers, and even undergo a sort of "babbling" practice phase like human infants and baby songbirds do (2). Knörnschild wants to track how communities of bats form and maintain local dialects over time. "If we can understand why vocal learning evolved repeatedly in different animals, and what selective pressures have caused it to evolve there, we can have better predictions on why it evolved in humans," she says.

But probing the biological mechanisms that control vocal learning will require invasive neuroscientific approaches. Weighing in at only a fraction of an ounce, the greater sac-winged bat is too small to carry implanted electrodes and other devices commonly used in neuroscience. At the University of California, Berkeley, Yartsev hopes that the heftier Egyptian fruit bat (*Rousettus aegyptiacus*) may be a better option.

For years, neuroscientists have focused on the sounds that bats make to echolocate. Only recently have a handful of researchers begun paying attention to bats' social vocalizations: in the Egyptian fruit bat, a repertoire of screeches, trills, and other cries that Yartsev calls their "vocabulary." "Our understanding of bat vocal learning behavior is lagging behind the massive amount of information we have about humans and birds," says Yartsev.

For many bat species, it's still unclear how robustly vocal learning occurs, but some early findings show promise. In 2015, researchers at Tel Aviv University reported that Egyptian fruit bat pups isolated from their parents are slow to develop mature, adult-like vocalizations, suggesting the babies rely on some form of learning. In fact, when isolated pups were exposed to recordings of select low-frequency adult calls, the pups developed a bias toward low-frequency vocalizations (13).

But such subtle changes fall short of demonstrating vocal learning, at least according to stricter standards. "I'm not yet totally convinced that they're full-fledged vocal learners," says Jarvis. According to Jarvis, the evidence so far suggests bats' imitative abilities may not be as advanced as those of songbirds. Yartsev hopes to settle these questions by teaching his bats to converse with computers.

In the first stage of the project, Cooper and his fellow bats have learned to winnow their spontaneous chatter down to a single call that they produce consistently to receive a reward. (Yartsev says each animal naturally settles on a preferred call after playing with the computer for a few weeks.) The exact call doesn't matter, as long as each animal reliably reproduces the same sound. In the next phase, the bats will hear their own call played back to them, and will be rewarded for answering the computer's call with theirs. The researchers will then gradually distort the computer's side of the conversation, doling out smoothie only when the bat matches the altered calls. Ultimately, Yartsev hopes to elicit sounds that no longer resemble "normal" bat calls, but are rather totally novel, learned vocalizations.

Yartsev thinks bats could offer something songbirds don't. Whereas zebra finches learn and sing a single song over and over, some bats appear to develop a vocabulary of sounds that they use flexibly in different combinations (14), similar to how human speech works. And there's another potential advantage, he says: zebra finches stop learning new vocalizations around puberty, but early evidence suggests that like humans, some bats exhibit certain types of vocal learning into adulthood (2).

To understand how this works, Yartsev's laboratory is beginning to map the brain areas and neural signals involved in the Egyptian fruit bat's social calls. And as Yartsev pursues neural circuits, Vernes, with whom he collaborates, is tracking down the genetic underpinnings of bat vocal learning.

Vernes's team is mapping the expression of *FoxP2* and *Cntnap2* (which is associated with a number of language disorders in children), and other genes in the brains of vocal learning bats, including the Egyptian fruit bat. With such maps in hand, Vernes hopes to target specific brain areas to switch individual genes on and off and decipher their precise roles in learning and communication.

More genetic leads may come from the BAT 1K initiative, an international consortium launched last November to sequence the genomes of the more than a thousand living bat species. It's early days, but researchers are already anticipating its results. "It's going to be awesome," says Knörnschild. "We can look for candidate genes and compare vocal and nonvocal learners." Bats are the second-largest group of mammals, after rodents, and thus represent a great deal of evolutionary diversity, an asset for such genetic studies. Genetic mechanisms shared between distantly related bat species are more likely to extrapolate to even more distant species, such as humans, says Vernes.

With so many parallel efforts underway, many believe that bats will be the ones to watch in the coming years. "In the next 10 years," says Fitch, "the bat could become every bit as valid and useful a model as birds for studying vocal learning."

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