to increase the breakdown of fat stores (triglycerides), or why whole-body energy expenditure is enhanced, given that a low concentration of leptin would decrease energy expenditure. Furthermore, it is not clear from the data whether the increase in food intake was an early event, preceding the elevation of plasma leptin, or whether it occurred later, as the authors propose.

Alternatively, the observed changes may reflect a loss of ACC2 activity in tissues other than muscle. Previous work by Abu-Elheiga (*6*) suggests that ACC2 in skeletal muscle is associated with mitochondria and that the malonyl CoA that it generates (versus that produced by ACC1) is an important regulator of carnitine palmitoyltransferase and fatty acid oxidation. Because some ACC2 may be present in cells in which ACC1 is the dominant isoform—such as pancreatic islets, human adipose tissue, and brain—it is possible that fatty acid oxidation and, secondarily, various signaling and downstream events are altered by loss of ACC2 in these or other tissues. Consistent with this notion are recent results suggesting that a pharmacologically induced increase in malonyl CoA in the hypothalamus both decreases the expression of neuropeptide Y and food intake and increases production of heat (thermogenesis) in obese mice (*5*). Thus, loss of ACC2 in hypothalamic neurons that regulate energy balance could alter energy intake and expenditure by modulating the production of malonyl CoA or another key metabolic intermediate. ACC has been detected in select neurons of the brain, notably in the arcuate nucleus of the hypothalamus (*5*), although which ACC isoforms are present in these neurons has not been determined.

Another very intriguing possibility is that the increased food intake and thermogenesis, and decreased adiposity of the ACC-deficient mice are caused by an increase in the expression of uncoupling protein–3 (UCP3), which exists exclusively in muscle (see the figure). UCP3 is a close relative of UCP1, which is found solely in brown fat, and similarly appears to be important for energy balance and lipid metabolism (*7*). Transgenic mice engineered to overexpress UCP3 in skeletal muscle are hyperphagic (that is, they overeat), yet they weigh less than their wild-type littermates (*8*). In addition, these mice show a striking decrease in fat tissue and have low glucose levels, as do the ACC-deficient mice. Intriguingly, activation of AMPK in muscle, whether by exercise or incubation with the AMPK activator 5-amino-imidazole 4-carboxamide riboside (AICAR), increases the expression of UCP3 within 1 to 2 hours (*9*). Because activation of AMPK in these situations is very rapid (seconds to minutes), as is ACC2 inhibition (*2*), it is conceivable that the increase in fatty acid oxidation in these muscles is responsible for increases in UCP3 mRNA and protein. That UCP3 expression in muscle is increased during starvation and other situations in which fatty acid oxidation is elevated is consistent with this notion, although in starvation, the increase in UCP3 is not sufficient to increase whole-body thermogenesis. The status of muscle UCP3 in ACC-deficient mice is eagerly awaited.

Finally, a number of studies provide evidence for cross talk between fat cells and other organs, most notably skeletal muscle and the central nervous system (*10*). Indeed, the discovery that adipocyte-derived molecules—such as leptin, tumor necrosis factor–α, gAcrp30 (*11*), and most recently resistin (*12*)—have systemic metabolic effects has firmly established the adipocyte as an endocrine organ. The results of Abu-Elheiga *et al.* and the findings in mice that overexpress UCP3 suggest that skeletal muscle could act in a similar manner to regulate whole-body energy homeostasis. Studies of altered signaling and gene transcription in muscle cells when fatty acid oxidation is increased or decreased, and an evaluation of whether and how these alterations are communicated to other organs are clearly in order.

The new work demonstrates that mice deficient in ACC2 have major alterations in systemic energy balance, with decreased body fat despite increased food intake. These findings provide important insights into the part played by malonyl CoA, the regulatory molecule produced by ACC2, in fatty acid oxidation in muscle and other cells. They also raise questions about the cellular sites and signal transduction pathways through which ACC2 exerts its newly identified systemic job. Whatever the mechanism, inhibition of ACC2 may be a plausible target for the design of new anti-obesity therapeutics.

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PERSPECTIVES: NEUROSCIENCE

The Song Does Not Remain the Same

Daniel Margoliash

You say tomato and I say to-mah-to. Why? This question may keep songwriters amused but has kept behavioral neurobiologists, who study speech development in humans and song development in birds, feverishly busy for decades. When humans learn to speak or to play an instrument they require guidance from a teacher, followed by a laborious period of trial-and-error imitation and iterative improvement. It is not yet clear how the teacher's instructions are represented in the neuronal circuitry of the brain and how they produce changes in behavior. Vocal learning in songbirds, a lengthy and complex process, is a valuable model for understanding how the human brain learns sequences of behavior from a teacher. But the complexities of song learning in birds have made systematic quantification of the process difficult. This, in turn, has hampered the matching of measures of altered neural activity with the evolving sounds produced by juvenile birds as they mature. On page 2564 of this week's *Science*, Tchernichovski and colleagues (*1*) combine a clever behavioral experiment on juvenile zebra finches with modern signal-analysis techniques to provide the first atomic description of song learning in birds. The results reveal several new and unsuspected features of vocal development.

Juvenile male zebra finches learn their song between 35 and 90 days after hatching (the sensitive period for vocal learning) by imitating the songs of adult male tutors. Zebra finch songs consist of complex sounds (syllables) separated by silent intervals. To obtain a baseline for the untutored vocal material of juvenile zebra finches, the investigators delayed song learning by preventing their exposure to adult males. Then, starting at 43 days of age, the juveniles were allowed to peck at a key, which triggered brief bouts of song from a small loudspeaker inside a model bird placed in the cage. Counterintuitively, limiting juveniles to brief periods of tutor-

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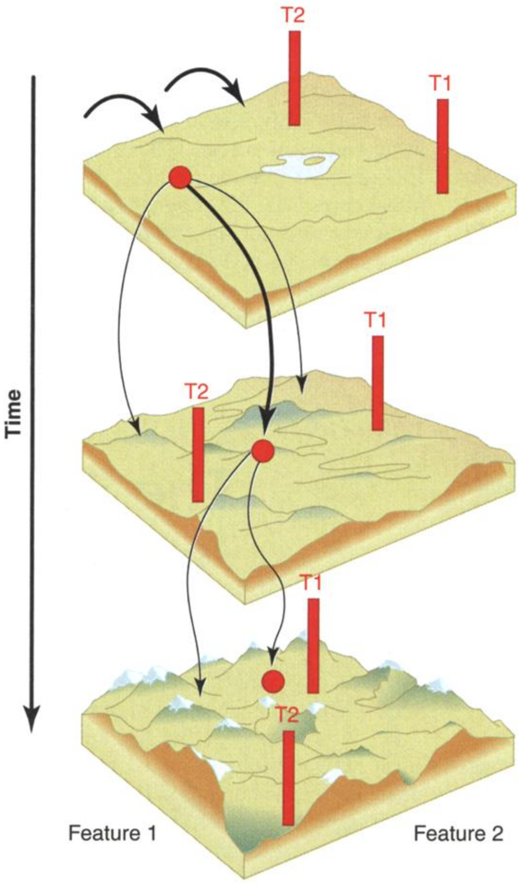
The author is in the Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637, USA. E-mail: dan@bigbird.uchicago.edu

ing by the model bird (only 28 seconds per day) actually improved the accuracy of learning (*2*). Although zebra finches rapidly acquire sounds under these conditions, the extent to which the abnormal delay in exposure to a tutor alters the normal developmental sequence of vocal learning is unclear. This could be tested in subsequent experiments in which, for example, juveniles could be exposed to a tutor's song earlier or later than 43 days of age or to a sequential series of songs from multiple tutors. Nevertheless, the elegant design of the new work brings the onset, speed, and accuracy of vocal learning under tight experimental control.

The trajectories of vocal development described by Tchernichovski *et al.* sometimes followed a path toward increasing the match between the surface acoustic features of the tutored and learned sound (the "direct" path), but often they did not. For example, when a juvenile tried to copy a tutor's sound with one of his own that was higher in pitch (frequency), the juvenile often increased the fundamental (base) frequency of his sound over a period of days in a direction away from the target, and then suddenly doubled the period (halved the frequency) to achieve a match (the "indirect" path).

What does this imply about the neural pathways that underlie bird song development? In the brain, sound frequency is probably controlled by the rate and timing of bursts of electrical activity (spikes) emitted by neurons in the forebrain nucleus robustus archistriatalis (RA) during singing (*3*, *4*). The frequency of a vocalization depends on the degree to which specific muscles in the syrinx (vocal organ) contract (*5*). A simple model to explain the direct and indirect paths for vocal learning proposes that input to the RA (or to local circuits within the RA) switches between two modes. In one mode, a small difference between the current and desired fundamental frequency alters the pattern of spike bursts generated by RA neurons, which, through altering muscle contractions in the syrinx, drives the sounds produced toward the correct frequency. In the second mode, a larger difference between the current and desired fundamental frequency (which exceeds a threshold difference) triggers changes in RA spike patterns that drive the sounds produced away from the desired frequency. When juvenile zebra finches matched syllables with those of a tutor through an indirect path, Tchernichovski *et al.* found that the evolution of the pitch error over time was nonlinear, suggesting that in the second mode the error can be measured as a proportional change. Eventually the pitch error was sufficient such that an exact match could be achieved by doubling the period (halving the frequency). Only a small change in central coordination may be required for the syrinx to produce period doublings (*6*). The two-mode model is attractive because it is simple and can be easily instantiated in plausible neural machinery, and because it makes clear predictions that can be tested.

Learning may proceed by copying a tutor either directly or indirectly, or by choosing among a set of preordained choices. These are referred to as instructional and selectional processes, respectively, and both have been proposed to describe vocal learning in birds (*7*, *8*). Tchernichovski *et al.* describe the earliest phase of song development as "generative," because they observed that as structure to the juvenile's song emerges, measures of vocal behavior increase in diversity. Clearly, the generative phase of song development could result from instructional processes, but it could also result from selectional processes (see the figure). Tchernichovski *et al.* argue that the selectional model should result in a decrease, not an increase, in the diversity of vocal behavior. But, as the greatest advances in song learning take place rapidly in the initial generative phase, any effects of selection might be hard to detect, especially with the limited vocal records that the authors collected. One cannot confidently rule out the possibility that during the generative phase, a group of pre-existing neural patterns representative of different vocalization prototypes are selected. These then compete and differentiate during development to achieve a match with the target song. The complex trajectories of continuity, pitch, and Wiener entropy (a measure of tonality) that the investigators describe for the one bird extensively sampled in the days after tutoring are not inconsistent with the selectional model, nor obviously predicted from the instructional model. Learning may involve a combination of both selectional and instructional processes (see the figure). Ultimately, we will need to know how different pre-existing neural patterns are represented in the brain to distinguish between these processes.



A new song to sing. The three landscapes represent three time points during vocal learning. Each landscape has an energy surface containing two of *N* features that specify a vocalization. The landscapes are the result of genetic and epigenetic influences, and change with environmental influence (bold arrows). The positions of vocal targets (T1, T2) also change over time. At each time point, the position of a vocal prototype (red dot) can change but is constrained by the landscape (thin arrows). Over time, learning implies that the prototype moves closer to target T1. Different models imply different constraints on this general scheme. In an instructional model, the landscapes develop over time, strongly influenced by the environment. In a selectional model, the environment influences which of a limited number of landscapes is chosen at each moment in time. The landscape changes slowly relative to the movement of the prototype, but in the initial (generative) phase of learning, the landscape changes more rapidly.

Doting parents can identify distinct periods when their babies seem to suddenly acquire new sounds. And so it was with the authors and their juvenile zebra finches. But, surprisingly these periods of rapid learning occurred on the day *after* tutoring, when the birds had slept (*1*). Recently, it has been observed that RA neurons burst spontaneously during sleep with spike burst patterns that closely match those produced by the same neurons when they participate in singing during the day (*9*). This spontaneous "replay" of singing during sleep by RA neurons suggests that sleep may possibly contribute to song learning by selecting among memories of songs performed during the day (*9*). This requires that the young birds have some vocal practice. But did the juveniles studied by Tchernichovski *et al.* modify their vocalizations on the first day of tutoring? It is unlikely that the birds failed to make any attempts at copying and practicing their tutor's song on the first day, although this might be difficult to detect with the relatively small sample of songs that were analyzed. If the birds did practice on the first day, they would have had the opportunity to rehearse their earliest attempts at imitation in their first period of sleep. The importance of sleep for learning could be tested by presenting the tutor material early in the day (giving birds considerable time to practice on the first day) or late in the day (when birds have had no time to practice before sleep). The ability to control the onset of rapid vocal learning with the Tchernichovski *et al.* protocol now makes this experiment feasible.


The zebra finch (*Taeniopygia guttata*).

In swamp sparrows (*10*) and indigo buntings (*11*), song learning has been described as following sequential modification of repeated structured syllables on the basis of acoustic similarity, and sometimes combining repeated structures (prototypes) to produce new syllables. The process described by Tchernichovski *et al.* is quite different. Young zebra finches often repeat a syllable prototype two or more times. The investigators observed that sequences of new syllables often developed from sequential repetitions of prototypes, a process they termed "in situ" differentiation. Remarkably, the prototypes could differ wildly from the target syllables of the tutor's song, so that sometimes in situ differentiation required major modifications of the leading or following components of the sequence. This is particularly surprising as better material (closer matches) was available to the birds in their untutored songs, although not in the proper sequence. How prevalent is this counterintuitive process? Here some caution is required. Because the experimental design delayed vocal learning, the young zebra finches had an unusually long time to practice in the early phases of song development before they were exposed to a tutor model. Nevertheless, in situ differentiation reinforces the conclusion that acoustic similarity is an insufficient predictor of vocal learning. Why might vocal learning follow such a seemingly difficult trajectory? The neural vocal pathway is hierarchically organized, with syllables and sequences of syllables programmed at higher levels than the RA (*3*, *4*, *12*). The dynamics of learning might be expressed differently at each level of the hierarchy. Once song sequences have developed, they may be relatively invariant, forming the scaffolding for subsequent morphological changes in individual components.

Behavioral analysis alone cannot solve the riddle of how birds learn to sing and neurobiological approaches alone cannot explain behavior. The Tchernichovski *et al.* study lays the groundwork for combining behavior and neurobiology to describe the mechanisms underpinning vocal learning in young birds. If we want to understand normal and abnormal vocal development, especially in inaccessible human brains, we must be prepared to take an integrative and comparative approach.

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PERSPECTIVES: ATOMIC FORCE MICROSCOPY

You May Squeeze the Atoms But Don't Mangle the Surface!

Alex de Lozanne

Imagine yourself at a fruit stand, with a blindfold over your eyes. You could probably identify various fruits by touching them, even using only one hand. Now imagine that you have a boxing glove over this hand and that you can only move it up and down with your arm outstretched. Identifying fruit is now far more difficult, but you may still be able to distinguish between a crisp apple and a ripe persimmon (without using your sense of smell!). You would do so by pressing on the fruit and feeling the reaction force on your glove. A hard fruit responds with a large force within a short distance, whereas a soft fruit gives in and responds with a smaller force.

On page 2580 of this issue, Lantz *et al.* (*1*) report an equivalent experiment at the atomic scale, performed by gently pressing the tip of an atomic force microscope (AFM) on the atoms of a silicon surface. There is one added complication, however: The tip is attracted to the surface at close range (think of sticky fruit). One therefore has to bring the tip to the surface and then quickly snap it back to prevent damage to the tip and/or surface should they stick together. Repetition of this motion produces an oscillatory movement, which is easier to control than a single dip. The oscillation of the AFM cantilever (your arm) changes frequency as the tip (your glove) interacts with the surface. Theoretical modeling is then used to extract information about the stiffness of the surface. This method enables Lantz *et al.* to measure the force resulting from an incipient bond between the tip atom and the surface atom and to distinguish between different types of silicon atoms (see the figure on the next page). For the first time, atomic resolution and a detailed measurement of these forces are obtained on the same surface.

The AFM was born in 1986, when a quintessential "back of the envelope" calculation by Binnig, Quate, and Gerber showed that it should be possible to measure the force between the last atom on a tip and individual atoms on the surface (*2*). Their first prototype showed a lateral resolution of 3 nm (*2*). Soon, beautiful AFM images with atomic resolution graced the covers of prestigious journals. It turned out, however, that these images never showed atomic-scale defects, such as a missing atom. It became clear that the images resulted from the interaction between many atoms on the tip with those on the sample. If the tip has a structure that is congruent with the surface structure,

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The author is in the Department of Physics, University of Texas, Austin, TX 78712, USA. E-mail: lozanne@physics.utexas.edu

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