have many inherent inaccuracies, this approach to understanding global photophysiology of phytoplankton should not be abandoned. We suggest that relating the space-based estimates to in situ measurements of chlorophyll fluorescence lifetimes will provide a pathway to understanding photobiological energy utilization and dissipation processes on a global scale. For example, the maximal average photochemical energy conversion efficiency (ϕ_p) at night in the global ocean, obtained simultaneously with our lifetime measurements, is 0.35 ± 0.11 (fig. S2). Given an average nighttime lifetime of 1.13 ns (Fig. 2), we deduce that thermal energy dissipation accounts for ~60% of the photosynthetically active quanta absorbed by phytoplankton globally. In contrast, under optimal growth conditions in the laboratory, an average phytoplankton cell uses ~65% of the absorbed quanta for photochemistry and dissipates <35% as heat. The fact that thermal dissipation of absorbed quanta by phytoplankton in the upper ocean is so high strongly implies that a large fraction of cells have impaired or nonfunctional PSII reaction centers and/or uncoupled photosynthetic antennae. We conclude that, although photochemical energy conversion to biomass in the oceans accounts for half of the global carbon fixed per annum, the overall energy conversion efficiency is relatively low and is limited by nutrient supply.

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NEURAL CIRCUITS

Inhibition protects acquired song segments during vocal learning in zebra finches

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Vocal imitation involves incorporating instructive auditory information into relevant motor circuits through processes that are poorly understood. In zebra finches, we found that exposure to a tutor's song drives spiking activity within premotor neurons in the juvenile, whereas inhibition suppresses such responses upon learning in adulthood. We measured inhibitory currents evoked by the tutor song throughout development while simultaneously quantifying each bird's learning trajectory. Surprisingly, we found that the maturation of synaptic inhibition onto premotor neurons is correlated with learning but not age. We used synthetic tutoring to demonstrate that inhibition is selective for specific song elements that have already been learned and not those still in refinement. Our results suggest that structured inhibition plays a crucial role during song acquisition, enabling a piece-by-piece mastery of complex tasks.

Humans (*1*) and several other animal species (*2–4*) learn motor sequences by imitation. In the observer, a sensory percept must inform relevant motor circuits involved in the generation of the target behavior, but little is known about the neural mechanisms underlying this process. We address this issue in the male zebra finch, which acquires its courtship song by listening to (movie S1) (*5*) and imitating (*6–9*) a tutor. The forebrain nucleus HVC acts as an important sensorimotor interface because it receives direct connections from higher-order auditory centers (*10–12*) and generates commands essential for song production (*13–15*).

In the juvenile zebra finch, tutor song exposure influences structural plasticity within HVC (*16*), a process that is thought to be crucial for song imitation (*17*). The tutor song has also been shown to drive network activity within HVC (*18*), but the responses of individual HVC premotor neurons during observational learning had not yet been explored.

We performed intracellular recordings in identified HVC neurons projecting to the robust nucleus of the arcopallium (RA) of 10 awake juvenile zebra finches during exposure to their tutor song. In 13 out of 29 of these HVC premotor neurons, tutor song playback caused spiking activity (Fig. 1, A to C). In those neurons, the timing of the evoked spikes was often highly precise across trials (see supplementary materials), demonstrating that exposure to the tutor song is sufficient to drive patterned spiking activity within HVC and may serve an instructive role for the developing HVC premotor circuit. We also observed reliably timed tutor-evoked spiking in RA (18 neurons in

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three birds) (fig. S1) that was presumably driven by HVC. In contrast, tutor song playback did not evoke a suprathreshold response in HVC premotor neurons of awake adult zebra finches (Fig. 1D, 0 of 24 cells in seven birds) and had only a minimal impact on subthreshold activity in those neurons (Fig. 1, D to F).

To directly investigate sensory-evoked synaptic events in HVC premotor neurons in awake zebra finches, we used in vivo whole-cell voltage clamp recordings (fig. S2). Using a fluorescent retrograde tracer injected into RA, we targeted HVC premotor neurons with two-photon microscopy guidance. In both juveniles (25 cells in 7 birds) and adults (15 cells in 5 birds), excitatory events could be evoked by the tutor song (fig. S3); this suggests that the observed lack of spiking in the adult bird is not explained by a decrease in the strength of sensory afferents from auditory projections to HVC. Our results seemed inconsistent with previous findings in which HVC projection neuron spiking could be reliably driven by song playback in urethane-anesthetized adult zebra finches (19). Because urethane has been shown to act as an antagonist for GABAergic transmission (20), we reasoned that synaptic inhibition might suppress tutor song–evoked excitation in HVC of awake adult zebra finches. To test that hypothesis, we locally infused a GABA$_A$ antagonist (gabazine) and recorded HVC premotor neurons during tutor song exposure in adults (Fig. 1G). Once local inhibition was attenuated, HVC premotor neurons exhibited tutor song–evoked patterned spiking responses (Fig. 1, G to I) similar to that seen in juvenile zebra finches (Fig. 1, A to C). This finding indicates that inhibition can effectively silence sensory inputs

onto HVC premotor neurons in the adult zebra finch.

Local circuit interneurons are likely to be the sole source of inhibition in HVC (21), and they exhibit song selective auditory responses in the awake adult zebra finch (22, 23). Thus, the lack of a tutor song response in HVC premotor neurons in adulthood may be due to a stronger recruitment of the inhibitory network. To address this idea, we performed juxtacellular recordings of HVC interneurons during tutor song presentation in both juvenile (34 cells in 10 birds) and adult (15 cells in 4 birds) zebra finches (Fig. 2, A to D). Across all ages tested, interneurons increased their spiking activity to tutor song playback relative to a silent baseline period (fig. S4), and we observed that the tutor song could evoke precise spiking activity in some cases (Fig. 2B). When considering all data across both juvenile and adult zebra finches, however, we noticed no consistent age-related difference in the regularity of interneuron firing across trials (Fig. 2C). Because the song learning process has been shown to be highly variable across individuals (6) (fig. S5), we reexamined our database on the similarity of recent song recordings from each bird to the tutor song. We found that interneuron firing precision in response to the tutor song tended to correlate with the extent of acoustic similarity to that song (Fig. 2D).

Because HVC interneurons densely interconnect with HVC premotor neurons (24, 25), they are well poised to directly inhibit HVC premotor neurons during tutor song presentation. Using two-photon targeted voltage-clamp recordings in awake birds, we found that inhibitory currents onto HVC premotor neurons were often reliably

evoked by tutor song playback (Fig. 2, E to H, and fig. S6). Consistent with our results concerning HVC interneuron firing, we found no evidence for an age-related change in the regularity or strength of tutor song–evoked inhibition onto HVC premotor neurons (fig. S7). Additionally, we found no age-related change in the amplitude or frequency of spontaneous inhibitory events (fig. S8) and a developmental decrease in the amount of current needed to hold premotor neurons at 0 mV, which could reflect a downregulation of tonic inhibition. However, in both juvenile and adult zebra finches, we found that the inhibitory charge, event frequency and amplitude, and regularity of inhibition across trials were significantly correlated with song imitation accuracy (Fig. 2, E to L, and fig. S6). These results demonstrate that the maturation of sensory-evoked inhibition in HVC matches the bird's learning progress rather than its developmental stage.

What function might this inhibition serve? We hypothesized that precisely timed inhibition in HVC could selectively target portions of the song that have been adequately learned, thereby suppressing the effect of sensory inputs on premotor neurons during those times and preventing further plasticity in motor output. We tested two predictions stemming from this idea. One prediction is that all premotor neurons should receive the inhibitory signal synchronously, which would allow for robust suppression of sensory inputs on the entire premotor system. A second prediction is that the global inhibitory signal should vary in strength as a function of how well each segment of the song has been learned, with stronger inhibition associated with better-learned segments. To test the first prediction, we considered 11 cases

Fig. 1. Responses of HVC premotor neurons to the tutor song are developmentally suppressed. (**A**) Example intracellular recording from an HVC premotor neuron in an awake juvenile zebra finch during tutor song presentation (sonogram frequency: 0.5 to 7.5 kHz). Below is a spike raster plot showing seven repetitions from the same neuron; the shaded region indicates the time of tutor song exposure with an additional 50 ms after the end of the song. (**B**) Firing rate of HVC premotor neuron firing in juvenile zebra finches [silence, 1.7 ± 2.7 Hz; tutor, 2.1 ± 3.3 Hz; $P = 0.114$, repeated-measures analysis of variance (ANOVA)]. (**C**) Spiking precision (see materials and methods) of HVC premotor neuron firing in juvenile zebra finches (silence, 0.0 ± 1.1; tutor, 3.0 ± 1.2; $P = 0.007$, repeated-measures ANOVA). (**D**) Example HVC premotor neuron recording in an awake adult bird during tutor song presentation, with spike raster below. (**E**) Membrane potential variance of HVC premotor neurons was greater in juveniles (5.8 ± 5.3 mV^2) than in adults (2.3 ± 1.6 mV^2) ($P = 0.004$, Wilcoxon rank sum test); the shaded region denotes the 95% confidence interval. (**F**) Subthreshold precision in HVC premotor neurons
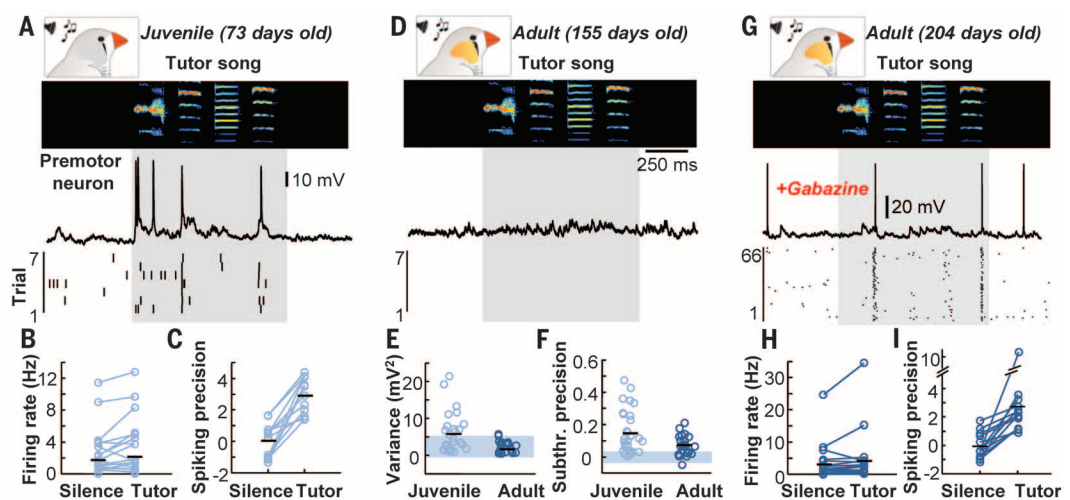
was greater in juveniles (0.16 ± 0.14) than in adults (0.08 ± 0.06) ($P = 0.025$, Wilcoxon rank sum test). (**G**) Tutor song responses from an HVC premotor neuron in an awake adult bird after local gabazine infusion (0.01 mM). (**H**) Firing rate during silence (3.6 ± 5.8 Hz) and during tutor song presentation (4.6 ± 8.2 Hz) ($n = 14$ neurons; $P < 0.001$, repeated-measures ANOVA) after local gabazine infusion. (**I**) Spiking precision was greater during the tutor song presentation (2.7 ± 2.5) than during silence (−0.1 ± 0.9) ($P = 0.016$, repeated-measures ANOVA).

in which multiple HVC premotor neurons (mean: 3.5 ± 1.3 neurons per bird) were recorded in the same bird (Fig. 3A). We found that tutor song–evoked inhibition was often highly correlated across neurons (Fig. 3B), and this effect was monotonically related to the degree of learning (Fig. 3D). The excitatory current profiles, however, seemed to be unique for each neuron (Fig. 3C) and did not significantly change with song

learning (Fig. 3E). Our results are consistent with a global entrainment of a motor circuit by an inhibitory network whose coherent song responsiveness may effectively suppress certain segments of the tutor song in a learning-dependent manner.

Zebra finches often learn individual song elements in series, focusing on specific passages at certain times (7). To test the second prediction of our model—that local inhibition in HVC is ac-

curately targeting the syllables of the tutor song that the zebra finch had learned—we used a previously established method for controlling the learning process of individual song syllables (7, 26). Zebra finches were first trained using a synthetic tutor that produced four concatenated copies of a single syllable "A" (Fig. 4A). Once the song "AAAA" was learned, the tutoring paradigm was altered to introduce an additional syllable
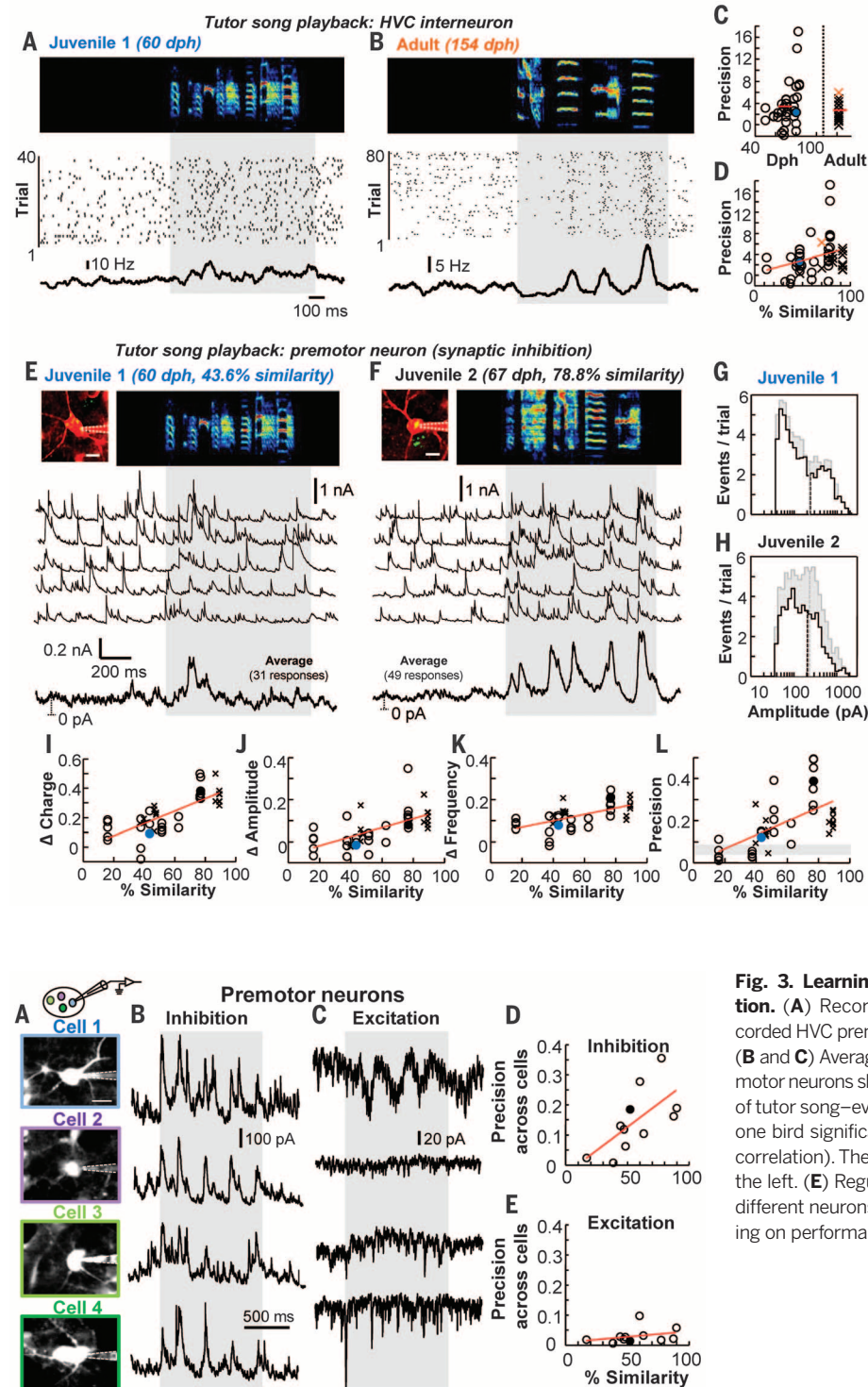

Fig. 2. Tutor song–evoked inhibition strengthens and sharpens with improved song performance. (**A** and **B**) Awake spiking activity of example HVC interneurons recorded in a juvenile (A) and an adult (B) bird during silence and tutor song presentation dph, days post-hatch. (**C**) Across the population, the precision of HVC interneuron firing did not differ between juveniles (3.8 ± 4.0 Hz) and adults (2.9 ± 1.7 Hz) ($P = 0.51$, Wilcoxon rank sum test). (**D**) Spiking precision of HVC interneurons depending on performance ($P = 0.056$, linear mixed-effect model). The solid circles and colored crosses represent data shown in the examples to the left. (**E** and **F**) Awake voltage-clamp recordings of inhibitory currents onto two HVC premotor neurons (images at top; scale bar, 10 μm) in response to a tutor song. For each cell, five single sweeps are presented as well as an average. The dotted line represents the distance from baseline (0 pA). (**G** and **H**) Amplitude histograms of detected inhibitory events during silence (black) and tutor song (gray) for juveniles 1 (G) and 2 (H). Mean of the amplitude distribution is indicated as a dashed vertical line. (**I** to **L**) Changes in tutor song–evoked inhibition onto HVC premotor neurons as a function of performance. Increasing similarity to the tutor song is associated with an increase ($P < 0.01$, linear mixed-effect model) in the inhibitory charge (I), amplitude (J), and frequency (K) of inhibitory events, and in the precision of inhibition across trials (L) (shaded region = 95% confidence interval). The solid circles represent data shown in the examples above.

Fig. 3. Learning is associated with synchronous network inhibition. (**A**) Recording schematic and two-photon images of four recorded HVC premotor neurons in the same zebra finch. Scale bar, 10 μm. (**B** and **C**) Average inhibitory (B) and excitatory (C) current traces of premotor neurons shown in (A) during tutor song presentation. (**D**) Regularity of tutor song–evoked inhibitory currents across different neurons within one bird significantly increases with learning ($P < 0.05$, Pearson linear correlation). The solid circles represent data shown in the examples to the left. (**E**) Regularity of tutor song–evoked excitatory currents across different neurons within one bird does not significantly change depending on performance ($P = 0.35$, Pearson linear correlation).

"B." Juvenile finches can eventually copy the new song "ABAB" (Fig. 4A), which leads to two distinct learning phases: an early learning phase in which "A" is performed well and "B" is performed poorly (Fig. 4, B and C), followed by a later learning phase in which both "A" and "B" are performed well (Fig. 4, D and E). We could exploit this artificially induced learning trajectory to address whether inhibition can be specifically directed toward portions of the song that have already been learned. In the early learning phase, when juveniles could perform syllable "A" well and not "B," interneuron firing (Fig. 4F) as well as inhibitory currents onto HVC premotor neurons (Fig. 4G) preferentially targeted the learned syllable (Fig. 4, H and I). Zebra finches at the later learning stage, which produced a good copy of both "A" and "B," showed equivalent interneuron firing and synaptic inhibition across both syllable types (Fig. 4, J to M). In contrast, excitatory currents did not change their relative timing across learning conditions (fig. S9).

Our results show that the activity of a motor circuit can be directly driven by sensory afferents during song learning. Specifically, exposure to the tutor song can elicit precise spiking in HVC premotor neurons of the juvenile bird (fig. S10A). This result is reminiscent of the "mirroring" previously observed in mammalian motor systems (27) as well as in other songbird species (28) in which motor neurons respond to actions that are observed in others, but the temporal similarity between the tutor song–evoked firing patterns and singing-related activity in individual HVC neurons of the juvenile remains unknown.

A previous experiment (17) demonstrated the necessity of tutor song–dependent HVC dynamics during vocal learning. In that study, juvenile zebra finches were unable to imitate the tutor when HVC activity was optogenetically scrambled during the presentation of the tutor song. These results are consistent with the idea that precise sensory-driven activity may have a pivotal role in establishing song-related premotor sequences.

We demonstrated a loss of auditory responsiveness in HVC of the adult bird, but we were able to use GABA antagonists to unmask tutor song–evoked spiking, thereby highlighting the role of inhibition in the suppression of these responses. Auditory-evoked responses were suppressed in all adults, even those that poorly copied the tutor song, indicating that the phasic inhibitory currents that are central to this study are not the only factor mediating this phenomenon. We did not find an age-related increase in tonic inhibition, which could have explained these results. Future studies could investigate the role of other developmentally regulated factors, such as intrinsic neuronal properties or chloride reversal potential, that could contribute to the further suppression of sensory inputs during development.

We also found that inhibition during song learning can precisely target specific portions of the song that have already been mastered (fig. S10B). As a result, certain components of the HVC sequence representing unlearned aspects

of the song may be left "exposed" to the influence of the incoming auditory stream. These neurons may then fire in a patterned way in response to the tutor song until an appropriate behavior is established. After the song has been learned completely, inhibition can shield HVC premotor neurons from the impact of the tutor song (fig. S10C). We do not yet understand the mechanisms that compare the current song performance to the tutor song and then transform this information

into a change in inhibition throughout learning. This process is likely to be primarily mediated through an increase in the regularity of inhibitory neuron firing across trials, which is driven from inputs from higher-order auditory centers (10–12).

In sensory systems, inhibitory network maturation can result in the closure of critical periods (29). However, this procedure is strongly dependent on the developmental stage of the animal,

Fig. 4. Inhibition accurately targets learned portions of the tutor song. (**A**) Schematic of the training procedure. (**B**) Example syllables produced by a juvenile during an early learning phase in which syllable A is copied with 82.5% similarity and syllable B is copied with 48.8% similarity. (**C**) Syllable performance for eight individuals in their early learning stage singing a good copy of A (77.9 ± 4.7% similarity) and a poor copy of B (42.2 ± 7.6% similarity). (**D**) Example syllables produced by a juvenile during a late learning phase in which both syllable A and B are copied well (85.7% and 88.9% similarity, respectively). (**E**) Syllable performance for four individual adults in their final learning stage singing a good copy of A (84.6 ± 1.9% similarity) and B (89.7 ± 2.2% similarity). (**F** and **G**) HVC interneuron activity (F) and inhibitory currents onto an HVC premotor neuron (G) during ABAB presentation recorded in two juvenile zebra finches performing a good copy of A (similarity: 75.6% and 84.6%, respectively) and a poor copy of B (similarity: 36.0% and 44.7%). The red and blue horizontal lines represent periods in which either the interneuron firing rate or HVC premotor neuron inhibition exceeds a 95% confidence interval. (**H** and **I**) Percentage of time that interneuron firing rates (n = 18 cells) (H) or HVC premotor inhibition (n = 10 cells) (I) exceeded the 95% confidence interval threshold across a population of four and five birds, respectively, that copied syllable A well and B poorly. (**J** and **K**) HVC interneuron activity (J) and HVC premotor neuron inhibition (K) during ABAB presentation recorded in a juvenile zebra finch performing a good copy of syllables A (similarity: 85.7% and 79.8%) and B (similarity: 88.9% and 94.9%). (**L** and **M**) Percentage of time that interneuron firing rates (n = 14 cells) (L) or HVC premotor inhibitory current amplitudes (n = 8 cells) (M) exceeded the 95% confidence interval threshold in two birds, respectively, that copied syllables A and B well.
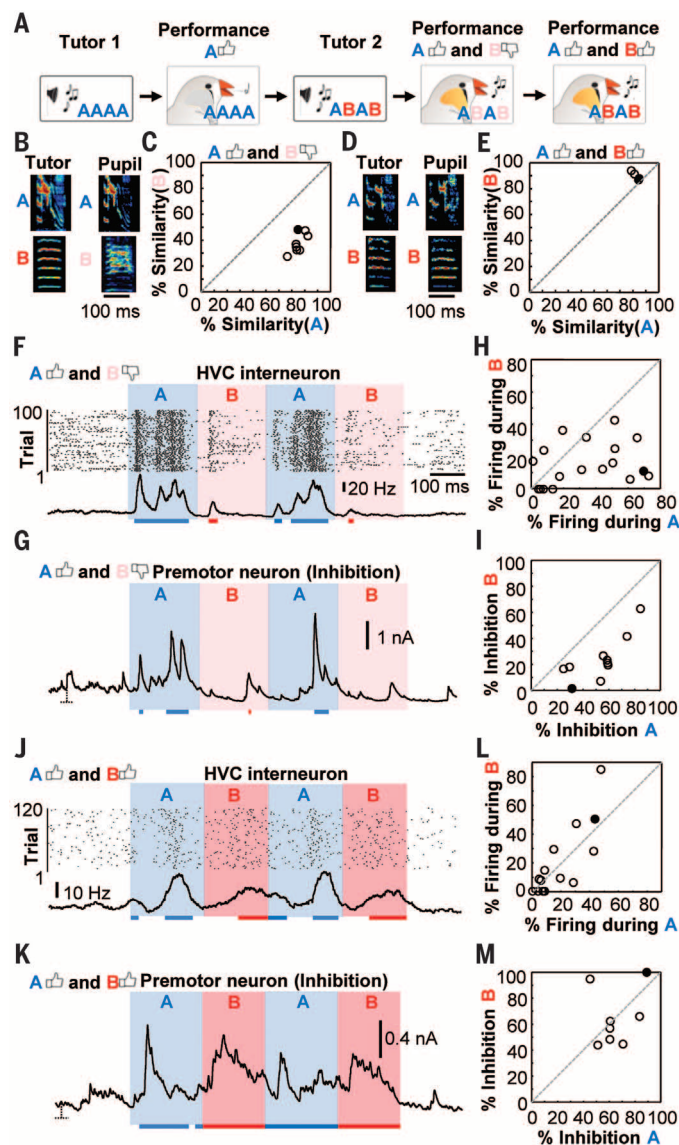
whereas the inhibitory network changes observed in HVC are correlated not with age but with song performance (fig. S10C). Additionally, because the extent of tutor imitation is variable across birds and even within the span of a single bird's song, the maturation of HVC inhibition proceeds in a self-directed, nonuniform manner. This stands in stark contrast to sensory systems, where inhibitory maturation primarily relies on external factors such as visual experience (30–32). Despite these differences, our findings offer the opportunity to potentially enable latent afferent streams to engage with motor circuits through the manipulation of local inhibition. Using this approach, we may help to extend (29) or reopen critical periods (33) in order to rebuild or refine skilled behaviors throughout life.

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MUSCLE PHYSIOLOGY

A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle

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Muscle contraction depends on release of Ca^{2+} from the sarcoplasmic reticulum (SR) and reuptake by the Ca^{2+}adenosine triphosphatase SERCA. We discovered a putative muscle-specific long noncoding RNA that encodes a peptide of 34 amino acids and that we named dwarf open reading frame (DWORF). DWORF localizes to the SR membrane, where it enhances SERCA activity by displacing the SERCA inhibitors, phospholamban, sarcolipin, and myoregulin. In mice, overexpression of DWORF in cardiomyocytes increases peak Ca^{2+} transient amplitude and SR Ca^{2+} load while reducing the time constant of cytosolic Ca^{2+} decay during each cycle of contraction-relaxation. Conversely, slow skeletal muscle lacking DWORF exhibits delayed Ca^{2+} clearance and relaxation and reduced SERCA activity. DWORF is the only endogenous peptide known to activate the SERCA pump by physical interaction and provides a means for enhancing muscle contractility.

Intracellular Ca^{2+} cycling is vitally important to the function of striated muscles and is altered in many muscle diseases. Upon electrical stimulation of the myocyte plasma membrane, Ca^{2+} is released from the sarcoplasmic reticulum (SR) and binds to the contractile apparatus triggering muscle contraction (1). Relaxation occurs as Ca^{2+} is pumped back into the SR by the sarco-endoplasmic reticulum Ca^{2+} adenosine triphosphatase (SERCA). SERCA activity is inhibited by the small transmembrane peptides phospholamban (PLN), sarcolipin (SLN), and myoregulin (MLN; also known as MRLN) in vertebrates and by sarcolamban A and B (sclA and sclB) in invertebrates, which diminish sarcoplasmic reticulum (SR) Ca^{2+} uptake and myocyte contractility (2–7).

Recently, we discovered the small open reading frame (ORF) of MLN within a transcript annotated as a long noncoding RNA (lncRNA) (4). We hypothesized that a subset of transcripts currently annotated as lncRNAs may encode small proteins that have evaded annotation efforts, a notion supported by recent proteomic analyses (8–10). To identify potential peptides, we searched presumably noncoding RNA transcripts for hypothetical ORFs using PhyloCSF; this method uses codon substitution frequencies (11). From these transcripts, we discovered a previously unrecognized ORF of 34 codons within a muscle-specific transcript, which we call dwarf open reading frame (*Dworf*) (fig. S1). The *Dworf* RNA transcript is annotated as NONCODE lncRNA gene NONMMUG026737 (12) in mice and lncRNA LOC100507537 in the University of California,

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Editor's Summary

Fixation on learned syllables

Zebra finches learn their beautiful songs by listening to other zebra finches. Vallentin *et al.* observed zebra finch brains as learning proceeded, only to find that inhibition of the neuronal circuits was critical to fixating on learned sequences. For song syllables that have been adequately learned, the inhibitory neurons fired coherently. Song syllables not yet learned failed to produce this flag. Thus, it is the learning process that alters the neuronal circuits. The inhibitory neuronal firing, not the age of the bird, locks in the learning.

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