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A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors

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In songbirds, as in mammals, basal ganglia-forebrain circuits are necessary for the learning and production of complex motor behaviors; however, the precise role of these circuits remains unknown. It has recently been shown that a basal ganglia-forebrain circuit in the songbird, which projects directly to vocal–motor circuitry, has a premotor function driving exploration necessary for vocal learning. It has also been hypothesized that this circuit, known as the anterior forebrain pathway (AFP), may generate an instructive signal that improves performance in the motor pathway. Here, we show that the output of the AFP directly implements a motor correction that reduces vocal errors. We use disruptive auditory feedback, contingent on song pitch, to induce learned changes in song structure over the course of hours and find that reversible inactivation of the output of the AFP produces an immediate regression of these learned changes. Thus, the AFP is involved in generating an error-reducing bias, which could increase the efficiency of vocal exploration and instruct synaptic changes in the motor pathway. We also find that learned changes in the song generated by the AFP are incorporated into the motor pathway within 1 day. Our observations support a view that basal ganglia-related circuits directly implement behavioral adaptations that minimize errors and subsequently stabilize these adaptations by training premotor cortical areas.

A.S.A. contributed new reagents/analytic tools; A.S.A. and M.S.F. analyzed data; and A.S.A. and M.S.F. wrote the paper.

The authors declare no conflict of interest.

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conditional feedback protocol. Spectrogram of targeted syllable (green curve). (B) The pitch distribution of the targeted region (green curve). (C) The spectral content of the targeted syllable played to the bird (blue arrows, AFP). To induce learning, disruptive auditory feedback is played to the bird via a speaker implanted in the cranial airsac that is internally continuous with the eardrum. Feedback signals are computed with a microphone on the head. HVC, proper name; RA, robust nucleus of the archicortex; X, Area X (proper name, homologous to the basal ganglia); DLM, dorsolateral nucleus of the medial thalamus; nXllts, nucleus of the 12th nerve. (B) Schematic of conditional feedback protocol. Spectrogram of targeted syllable (Top), total duration 180 ms. A measure of pitch is computed continuously (Middle, black curve). Whenever the pitch falls above a threshold (blue region) white noise is played to the bird (Bottom). The threshold is position in the center of the pitch distribution of the targeted region (green curve). (C) (Left) The average pitch over the course of several hours that dramatically reduced the amount of auditory feedback “error.” Does the AFP make a premotor contribution to these adaptive changes in pitch? To address this question, we used tetrodotoxin (TTX) to transiently inactivate LMAN during learning (Fig. 2A and Figs. S3 and S4). If premotor signals from the AFP directly contribute to learned pitch changes, we would expect inactivation to cause an immediate regression of the learned change. In contrast, if the learned pitch changes are immediately implemented by synaptic plasticity in the motor pathway, we would expect the average pitch to be the same before and after LMAN inactivation. Each day, after the first h of conditional feedback, we infused either TTX or vehicle (on alternating days) into LMAN. Inactivation of LMAN with TTX produced an immediate change in pitch in a direction opposite that of the ongoing learning (Fig. 2B and E). 16.3 ± 2.2 Hz, n = 34 inactivations, P < 10−7, 2-tailed t test, also see Figs. S5 and S6). Inactivation most often resulted in an increase in the amount of disruptive auditory feedback played to the bird (Fig. 2D, 30/34 inactivations, feedback power increased by a factor of 15 ± 6, P < 0.02, 1-tailed t test). In contrast, infusion of vehicle had no significant effect on average pitch (Fig. 2C and F, n = 34 infusions, P > 0.4). These observations suggest that the AFP contributes to vocal output by biasing syllable pitch in a direction that reduces experimentally imposed error.

In the remaining text, we refer to the pitch of the targeted syllable generated with LMAN intact and LMAN inactivated as “LMAN(+) pitch” and “LMAN(−) pitch” respectively. The motor pathway encodes a stereotyped motor program for singing that operates independently of the AFP (7, 8, 15, 16). Thus, LMAN(−) pitch reflects this motor-pathway-encoded version of the targeted syllable. Furthermore, the contribution of the AFP to the pitch of the targeted syllable can be quantified as LMAN(+)/LMAN(−) pitch, which we refer to as “AFP-dependent bias,” or more briefly as “AFP bias.” Across multiple days of conditional feedback, we observed large accumulating changes in the pitch of the targeted syllable (Fig. 1D). Are all of these changes due to accumulating AFP bias, or is there also a contribution from AFP-independent mechanisms, such as plasticity in the motor pathway? To answer this question, we examined whether, over the course of the experiment, LMAN(−) pitch deviated from the baseline pitch sung before the first day of conditional feedback. We found that LMAN(−) pitch exhibited substantial deviations from baseline (Fig. 3A and B, range = 121 ± 5 Hz SD). In fact, deviations in LMAN(−) pitch closely tracked the deviations of LMAN(+) pitch (Fig. 3C and D, n = 34, r² = 0.85, slope = 1.08 ± 0.08), indicating that during the course of our experiments, AFP-independent mechanisms contributed substantially to the expression of learned changes in pitch. In contrast, the magnitude of the AFP bias was not correlated with how far the pitch deviated from baseline (Fig. 3E, P > 0.3, slope not different from 0), indicating that continued learning in the same direction over...
multiple days was not a result of increasing AFP-dependent bias, but rather an increasing AFP-independent contribution.

Consistent with this result, we found that LMAN inactivation caused the pitch of targeted syllable to regress to near the pitch in the first morning songs. That is, the size of the AFP bias was correlated with the size of the pitch shift that was learned during the morning before inactivation (Fig. 4 A and B, \( n = 34, r^2 = 0.73 \), slope = 0.98 ± 0.11, \( P < 10^{-8}, \) slope > 0). Taken together, our findings thus far suggest that the AFP makes a direct contribution to learned pitch changes, but that the size of the AFP contribution is limited and is proportional to the amount of learning that occurred within the last day. In addition, during multiple days of learning, the majority of the accumulated deviations in pitch from baseline are encoded in the motor pathway.

The syllable pitch encoded in the motor pathway is highly plastic, as evidenced by the large changes in LMAN(−) pitch between successive inactivations (Fig. 3 B). We tested the hypothesis that these plastic changes are predicted by the amount of AFP bias observed during some preceding interval. Plastic changes in the motor pathway were assessed as the difference between successive LMAN(−) syllable pitch measurements (Fig. S4). Because LMAN inactivations were carried out every other day, this measure reflects the change over a 2-day interval. The total AFP bias was also calculated in 2-day intervals as a sum over consecutive days. AFP bias was directly measured on TTX days, and was estimated on vehicle days as the total amount of learning that occurred on that day—motivated by our finding that the AFP bias is approximately equal to the amount of learning that occurred before TTX infusion (Fig. 4).

We started by examining the relation between motor pathway plasticity and AFP bias 1 day earlier. Specifically, we find that plastic changes in the motor pathway over a 2-day interval (e.g., between days \( n - 2 \) and \( n \)) are strongly correlated with the sum of the AFP bias measured on day \( n - 2 \) and estimated on day \( n - 1 \) (Fig. S4 A–C, \( r^2 = 0.93, \) slope = 0.99 ± 0.06). This correlation is further supported by examining variations in the amount of learning. On up days, a large AFP bias was typically followed by a large change in the motor pathway, and a small AFP bias was followed by a small change. This same relation held for down days, but the signs of the quantities were negative. To combine all of the data, we inverted the sign of the data for down days, but the signs of the quantities were negative. To combine all of the data, we inverted the sign of the data for down days, but the signs of the quantities were negative.

We next examined the temporal specificity of the relation between AFP bias and subsequent plasticity in the motor pathway. Do variations in AFP bias only correlate with changes in LMAN(−) pitch on the next day, or do they also correlate with motor plasticity on the same day, or 2 days later? We find that variations in AFP bias were not as strongly correlated with variations in motor plasticity observed on the same day (lag = 0, \( r^2 = 0.18 \), or 2 days later (Fig. 5 D and E, lag = −2: \( r^2 = 0.08 \), also see Fig. S7). Of course, a careful examination of the temporal relation between AFP bias and plasticity in the motor pathway will require experiments in which LMAN inactivations are carried out at a finer time resolution. Nevertheless, these results lends further support to the hypothesis that AFP bias may be subsequently consolidated in the motor pathway by the end of the following day.

AFP bias was not only correlated with plastic changes in the motor pathway over the next day, but these quantities have the same magnitude. The change in LMAN(−) pitch between successive measurements (average 16.7 ± 1.2 Hz/day) was not significantly different from the size of the estimated AFP bias at a lag of minus 1 day (average 15.1 ± 1.7 Hz, \( P > 0.11, \) paired \( t \) test, see Methods), suggesting a remarkable correspondence between the mechanisms that underlie AFP bias and subsequent motor plasticity.

Discussion

Our experiments take advantage of the fact that, in response to an experimentally controlled association between syllable pitch and auditory error, a singing bird makes rapid corrective pitch changes (18). Applied over many days this protocol produced large accumulating changes in pitch. By inactivating the AFP during learning, we identified 2 contributions to the observed vocal plasticity. First, we found an AFP-dependent contribution that biases vocal output to reduce the probability and intensity
of imposed error. Second, we observed an AFP-independent contribution, likely due to plasticity in the motor pathway, that accounts for the majority of the accumulated pitch changes. We also found a remarkable correspondence between AFP-dependent bias and plastic changes in the motor pathway observed within the next day. Thus, our results establish the time scale on which adaptive changes in vocal output become encoded in the motor pathway and expressed in an AFP-independent manner.

Our findings suggest a possible role for AFP-dependent bias during natural vocal imitation. It will be important to determine whether during natural song learning, the AFP biases motor output to improve the match between auditory feedback and tutor song. This could be examined by carrying out LMAN inactivations during the rapid, early phases of vocal learning in juvenile songbirds. We anticipate that inactivation of LMAN in the evening would result in a regression of learned changes in the song that occurred during the hours before inactivation and furthermore would result in a decrease in similarity with tutor song.

There are several mechanisms by which the bias we observe could be dependent on AFP activity. The AFP could serve a permissive role. For example, it is possible that HVC drives bias, but that its expression is dependent on AFP input to RA. Alternatively, the AFP could serve a direct premotor role in generating bias. Given the premotor contribution of the AFP to generating vocal variability, we favor the interpretation that AFP-driven variability itself becomes biased, generating larger or more frequent variations in the direction of reduced vocal error.

The mechanistic role of the 3 AFP nuclei in generating bias remains to be determined. Striatal neurons have been shown to be involved in the rapid evaluation of rewarded associations (13) and in the coding of action-specific reward values (24, 25). One possibility is that “random” activity patterns in LMAN produce variability in vocal output and are evaluated by basal ganglia homologue Area X. The results of this evaluation could then be sent to LMAN (through pallidal-recipient thalamic nucleus DLM) to reinforce LMAN activity patterns that produced desirable vocal output, thereby resulting in bias. Area X receives an efference copy of activity in LMAN (26, 27) and HVC (28, 29), thus placing Area X in a position to evaluate exploratory activity in LMAN in the context of the ongoing song. It is not known, however, whether Area X receives evaluative feedback about song performance either from auditory areas (30) or from midbrain dopaminergic areas (31).

Trial and error, or reinforcement, learning requires both exploratory behavior and the evaluation of performance (32). Biased variability could subserve both of these functions. First, biased variability could make motor exploration more efficient by increasing the speed at which effective motor control parameters are discovered. Second, AFP bias could instruct plasticity in the motor pathway (21). Although we cannot rule out the possibility that AFP bias and motor plasticity are implemented by separate evaluative mechanisms, our finding that the amount of AFP bias on any 1 day is correlated with, and has the same
bien learning (33). However, several studies have shown that sleep may play an important role in the consolidation of learned skills (34–37). In particular, observations of sleep-replay activity in the motor pathway have led to the proposal that plasticity in this circuit occurs off-line (34), perhaps during sleep. Repeated brief inactivation of LMAN at several time points during the day would help distinguish these hypotheses.

Broadly speaking, our observations shed light on the function of basal ganglia–forebrain circuits in vertebrates, particularly the mechanisms by which practice of complex motor skills results in improved performance, and even more generally, the mechanisms by which goal-directed behaviors become entrained as highly stereotyped sequences or habits (5).

Materials and Methods

Subjects. Juvenile and young adult (age 77–182 dph) male zebra finches (Taeniopygia guttata) were used. Animals were selected for sufficient singing rates (~200 song bouts per day), and for songs containing a harmonic stack with a pitch unique within the song and between 500 and 2000 Hz. All procedures were approved by the Massachusetts Institute of Technology Committee on Animal Care. For surgical procedures, see SI Methods.

Song Recording. Birds were housed individually in custom sound isolation chambers. Singing was measured using a miniature microphone (WBHC-23910, Knowles Electronics, Inc, 0.08 g, Fig. S1A) attached to the bird’s head during surgery, providing measurement of song largely independent of position in the cage. Monitoring and recording of song was performed using custom MATLAB (Mathworks) software.

Conditional Auditory Feedback. The auditory signal perceived by the bird during singing was disrupted by playing broadband noise. This disruptive auditory feedback was generated using a hearing aid speaker (EM-23046-CX, Knowles Electronics, 0.21 g) and transmitted into the cranial air sac surrounding the cerebellum via an implanted speaker tube (Fig. S1). Sound levels were calibrated individually for each bird (see SI Methods). In this configuration, feedback did not distort the signal recorded by the head-mounted microphone, allowing uninterrupted monitoring of singing.

In our conditional feedback protocol, feedback was contingent on a measure of syllable pitch (18). This measure was computed using a set of finite-impulse response (FIR) filters implemented in custom software running on a digital signal processor (RX8, Tucker-Davis Technologies) (Fig. S2 and SI Methods). The measure of syllable pitch was used to calculate the loudness of the feedback noise relative to the loudness of the ongoing song vocalization. In this way, the bird could not escape feedback simply by singing louder (see SI Methods). The pitch threshold for feedback was constant during each day, and was set each morning to the average pitch of the targeted syllable measured at the end of the previous day. All feedback powers reported are calibrated acoustic power played into the cranial air sac.

Transient LMAN Inactivation. Custom reverse microdialysis probes were built using dialysis tubing (200-μm diameter) attached to a drug reservoir (Fig. S3 A and B), as described in ref. 17. Probes were implanted bilaterally into LMAN using stereotaxic coordinates (Fig. S3C). Inactivation was carried out by filling the reservoir and dialysist tubing with 25 μM TTX in PBS. This concentration was found to saturate the reduction of pitch variability resulting from inactivation of LMAN (from dose–response curve, Fig. S4C). After nominally 4 h, drug was removed by flushing the tubing and filling the reservoir with PBS. On vehicle days, the same procedures were carried out with PBS instead of TTX. Electro-physiological recordings in anesthetized birds (n = 2) confirmed that 25 μM TTX infusion completely suppressed electrical activity within 0.75 mm of the probe, thus fully encompassing LMAN. In contrast, activity persisted in medial MAN (1.05 mm from the probe) for the duration of the recording session, at least 1–1.5 h after infusion.

Experimental Design. The birds were housed individually in custom sound-attenuated chambers equipped with 10-channel commutators (SL-88, Dragonfly), and were maintained on a 12 h:12 h light–dark cycle. Conditional feedback was started once the birds consistently sang after infusion. TTX and vehicle were infused on alternate days, nominally 4 h (3.9 ± 1.0 SD) after the first morning singing. Before lights on, the FIR filters were updated so that the edge of the band of targeted pitches (i.e., the threshold) was placed approximately at the center of the pitch distribution at the end of the previous day (last 50 syllables, Fig. S2C). On the morning after a TTX day, the threshold was

Fig. 5. AFP bias is predictive of subsequent plasticity in the motor pathway. (A) Motor plasticity was assessed as the difference (∆m) in LMAN (−) pitch between successive inactivations (red dots), carried out every other day. Gray dots represent morning and preactivation syllable pitch. We examined the relation between motor plasticity and the estimated total AFP bias over a corresponding 2-day interval. The total was computed as the sum (β + β*) of the AFP bias on TTX days (β) plus the AFP bias on vehicle days (β*)—the latter estimated as the amount of learning that occurred during the day (see Fig. 4). (B) Scatter plot of motor plasticity (∆m) and estimated 2-day sum of AFP bias (β + β*; linear regression, red dashed line; slope = 0.99 ± 0.06, r^2 = 0.93). Time series of ∆m (red squares) and a 2-day running sum of estimated AFP bias 1 day earlier (black diamonds), as shown in A. (C) Scatter plots showing the correlation between ∆m versus the estimated 2-day sum of AFP bias at lags of −2, −1, and 0 days (linear regression, red dashed line; slopes = −0.08 ± 0.06, 0.61 ± 0.08, 0.23 ± 0.10 respectively; up and down days combined by inverting down days; see Fig. S7). A–C correspond to a lag of −1 day. (E) Correlation coefficients as a function of time lag (days). Errors bars are 95% confidence intervals.

magnitude, as the amount of plasticity in the motor pathway within the next day, favors a causal role for the AFP in driving this plasticity. Furthermore, it suggests a view in which plasticity in the motor pathway temporally integrates, or accumulates, a more rapidly learned motor signal expressed by the AFP. The precise time course and mechanism of consolidation into the motor pathway remains an open question. For example, it is possible that AFP bias actively drives plasticity in the motor pathway during singing by an on-line mechanism, such as Hebb-
centered to the average of the last 50 syllables before TTX infusion. See SI Methods for further details and statistics. Conditional feedback remained on throughout the entire day, including during and after drug infusion.

Data Analysis. Song was segmented into syllables based on song amplitude. The fidelity of the head-mounted microphone signal made segmentation highly reliable. Acoustic feature vectors were calculated for all segmented syllables (duration, amplitude, entropy, pitch goodness, and variance of the last 2 measures). Syllables were classified using these feature vectors and custom hand-clustering software. For each rendition of the targeted syllable, the time course of the pitch (Fig. S5) and feedback power were computed. By custom hand-clustering software. For each rendition of the targeted syllable, the time of infusion, and the final 50 renditions of the day. These values were used to quantify the changes in pitch: (i) during learning before infusion, (ii) as a result of infusion, and (iii) overnight. Overnight changes in pitch were quantified as the difference between the morning pitch and that of the final renditions of the preceding day; nights after TTX infusion were excluded from this analysis.

Baseline pitch was computed as the average pitch of the targeted syllable on the day before starting feedback.

The time series of motor pathway plasticity (Δm) and the estimated AFP bias (β̂) were plotted (Fig. S5C) as follows: The motor pathway plasticity plotted on day n was calculated as LMAN(−) pitch on day n minus LMAN(−) pitch on day n−2. These points appear every other day because LMAN was only inactivated on alternate days. The running 2-day sum of AFP bias plotted on day n was calculated as the sum of AFP bias on day n−2 and the AFP bias on day n−1. The 95% confidence intervals for the correlation coefficients in Fig. S5E were computed using nonparametric bootstrapping.

Histology. Animals were deeply anesthetized with pentobarbital and perfused with 4% paraformaldehyde (Sigma). Brains were removed from the skull and postfixed in 4% paraformaldehyde. Brains were sectioned parasagittally with a vibrating Microtome (100 µm thick, Vibratome 1000, TPI), and the location of the dialysis membrane was determined (Fig. S5C).

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