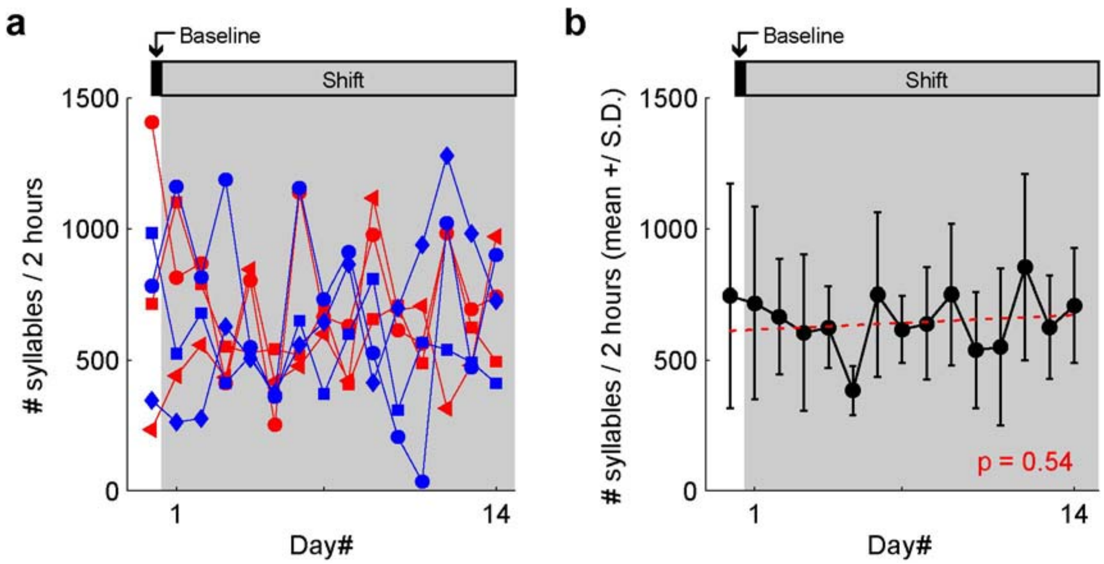
Pitch shifts do not alter the amount of song produced



Supplementary Figure 1: The number of syllables produced does not change significantly over the course of the shift epoch. **a**, Number of syllables produced during each day's analysis window (10am-noon) during the baseline and shift epochs. Color and symbol shape conventions as in Figure 3a in the main text. **b**, Mean +/- S.D. number of syllables, combined across experiments. No significant correlation between time and the amount of singing was observed (dashed red line, p=0.54).

Pitch shifts do not affect the syntax of song

We compared the syntax of song before and 14 days after the onset of pitch shifts to determine whether shifted auditory feedback led to changes in the ordering of song syllables. We found that the gross structure of song was not altered by pitch shifts, and that when more precise measures of syntax were used there were no greater changes in response to pitch shifts than were present in control conditions. As shown in Supplementary Figure 2, we constructed a syllable transition matrix for each bird's song both in the baseline epoch and at the end of the shift epoch. Each entry in the matrix describes the probability of one syllable following another. For example, in the baseline epoch in the example shown below, probability of syllable 'B' being followed by syllable 'C' was 0.61. We then subjected the transition matrices to two different analyses. To asses any changes to the gross structure of song, we first asked whether the most common (or "dominant") transition in the baseline condition was also the dominant transition following adaptation to a pitch shift. In each matrix shown below, the dominant transition (i.e. the transition in each column that has the highest probability) is shown in bold. We found that in all cases (45/45 syllables analyzed) the dominant transition in the baseline epoch was also the dominant transition following the feedback shift. This result indicates that on a gross level song syntax is not changed as a result of shifts in the pitch of auditory feedback.

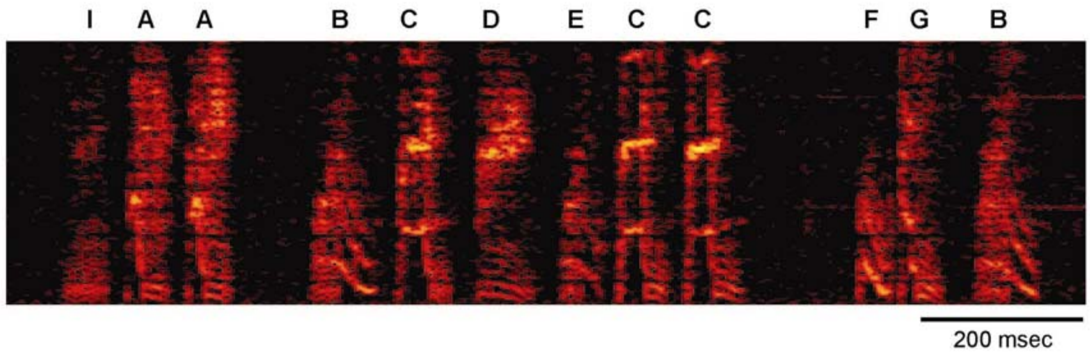
To investigate subtler syntax changes, we also compared the prevalence of changes in transition probability in control birds to that observed in birds experiencing pitch shifts. To do this, we asked whether each transition probability (e.g. each entry in the transition matrices shown below) changed significantly between the baseline and shift epochs (using a z-test for proportions at $p<0.05$ and a Bonferroni correction for multiple comparisons), considering every case in which a transition had a nonzero probability in either epoch. In a group of control birds that did not wear headphones, we found that 3% (3/106) of transition probabilities changed over a 14-day period. This proportion was not significantly different from that observed in pitch-shifted birds ($p=0.46$, z-test for proportions), in which 6% (7/124) of transition probabilities changed over the 14-day shift epoch. We therefore conclude that syntax is not significantly affected by the shifted auditory feedback used in our experiments.

Finally, among the 7 significant changes in transition probability, we asked whether a significant majority reflected increases or decreases in sequence entropy. Deafening adult birds and introducing auditory perturbations have both been shown to increase the entropy of syllable transitions (Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997; Sakata and Brainard, 2006). For each transition, if the quantity

$$[-p_{shift}\log(p_{shift})] - [-p_{baseline}\log(p_{baseline})]$$

was greater than zero, then the entropy of that transition increased, and values less than zero represent decreases in entropy. We found that 29% (2/7) of changes in transition probabilities represented entropy increases and 71% (5/7) were entropy decreases. The ratio of increases to decreases was not significantly different from equality (chi-squared test, p=0.26).

a



b

Baseline

	I	A	B	C	D	E	F	G
I	0.42	0.10	0.34	0.22	0	0	0	0
A	**0.45**	**0.49**	0	0	0	0	0	0
B	0.02	0.41	0	0.01	0	0	0	**1.00**
C	0	0	**0.61**	0.34	0	**1.00**	0	0
D	0	0	0	**0.34**	0	0	0	0
E	0	0	0	0	**1.00**	0	0	0
F	0.12	0	0.04	0.09	0	0	0	0
G	0	0	0	0	0	0	**1.00**	0

+100 cent shift

	I	A	B	C	D	E	F	G
I	0.44	0.08	0.33	0.12	0.003	0	0	0
A	**0.53**	**0.49**	0	0	0	0	0	0
B	0	0.41	0	0	0	0	0	**1.00**
C	0	0	**0.65**	0.35	0.003	**1.00**	0	0
D	0	0	0	**0.35**	0	0	0	0
E	0	0	0	0	**0.99**	0	0	0
F	0.02	0.003	0.01	0.17	0	0	0	0
G	0	0	0	0	0	0	**1.00**	0

Supplementary Figure 2: Technique for quantifying changes in syntax. **a**, Segment of song showing the 8 syllables (IABCDEFG) produced by one of the birds in our study. **b**, Matrices describing the probability of transitions between syllables in the baseline epoch (left) and the last day of the shift epoch (right). The row and column of each matrix describes the probability with which the syllable indicated at the top of each column will be followed by the syllable indicated to the left of each row. For example, in the baseline epoch syllable "A" has a probability of 0.41 of being followed by syllable "B". In each column, the transition with the greatest probability is shown in boldface type.

References:

Okanoya, K. & Yamaguchi, A. Adult Bengalese finches (*Lonchura striata var. domestica*) require real-time auditory feedback to produce normal song syntax. Journal of Neurobiology 33, 343-356 (1997).

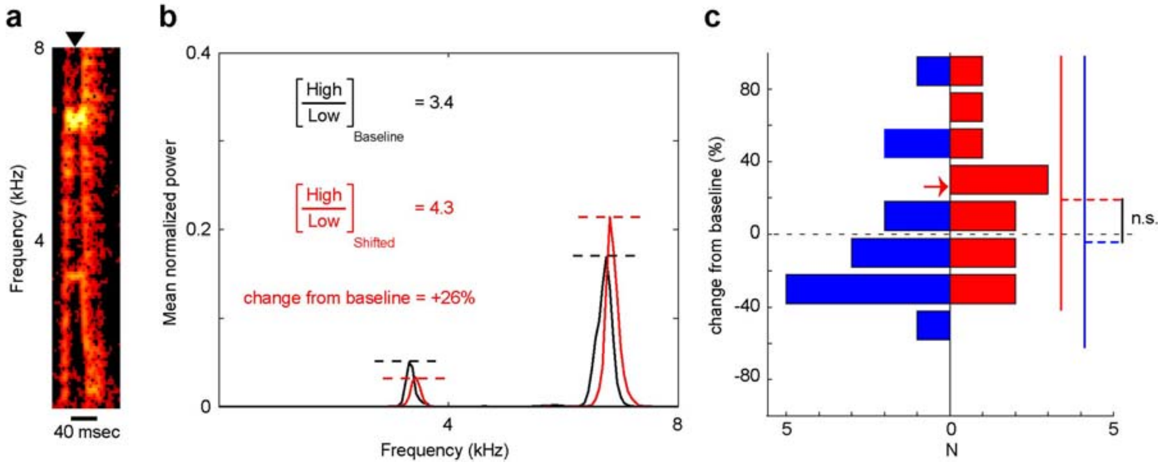
Sakata JT, Brainard MS Real-time contributions of auditory feedback to avian vocal motor control. J Neurosci 26:9619-9628 (2006).

Woolley, S. M. & Rubel, E. W. Bengalese finches Lonchura Striata domestica depend upon auditory feedback for the maintenance of adult song. J Neurosci 17, 6380-6390 (1997).

Pitch shifts do not consistently change spectral emphasis

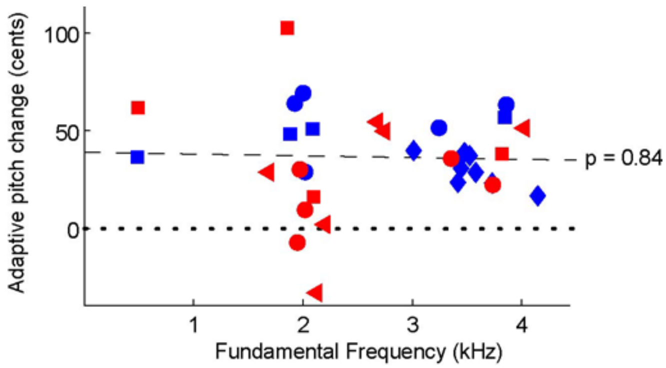
The analysis described in the main text shows that birds shift the fundamental frequency of syllables in the direction opposite the imposed feedback shift. However, birds might also change the relative acoustic power of each syllable's harmonics ("spectral emphasis"), presumably by changing the filtering properties of the upper vocal tract (for example, by emphasizing the higher harmonics in order to compensate for a downshift in the pitch of auditory feedback).

Our technique for measuring such changes is illustrated in Supplementary Figure 3 below. For each spectral frame, we computed the mean normalized power spectrum both during the baseline epoch and at the end of the shift epoch (black and red lines, Supplementary Fig. 3b). We then computed the ratio of the peak power at the highest and lowest measurable harmonics (dashed lines in Supplementary Fig. 3b). The example in Supplementary Figure 3 shows the change in mean power spectrum before and two weeks after the onset of a 100 cent downward shift in the pitch of auditory feedback. The ratio of peak power at the high and low harmonics increased 26% over the shift epoch, corresponding to an increased emphasis on the high harmonic. This datum is indicated with a red arrow in Supplementary Figure 3c. When this analysis was performed for all syllables, we found no significant difference in the change in spectral emphasis following upward or downward shifts in the pitch of auditory feedback ($p=0.16$, 1-tailed t-test). Together with our demonstration of significant adaptive changes in the pitch of song syllables, this result suggests that our feedback manipulations primarily drive changes in syringeal control as opposed to changes in upper vocal tract filtering.

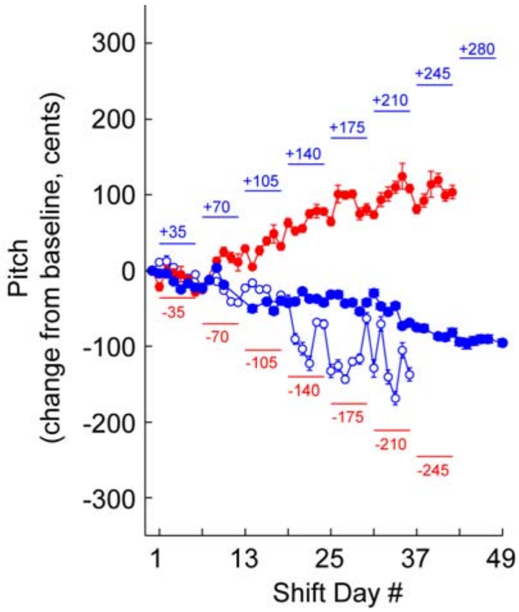


Supplementary Figure 3: Changes in spectral emphasis. **a**, Spectrogram of an example syllable. The black arrowhead indicates the spectral frame (measurement time) used to calculate the power spectra. **b**, Mean normalized power spectra of the example syllable in the baseline epoch (black) and during shift days 12-14 during a downward shift in the pitch of auditory feedback (red). As described in the text, we quantified the ratio of peak power (dashed lines) at the highest and lowest measurable harmonic (ratios shown in inset) and then quantified the change in this measure between the baseline and shift epochs. In this example, the "harmonic ratio" increased by 26%, corresponding to an increase in power at the high harmonic. **c**, Change in harmonic ratio for all spectral features, combined across all downward (red) and upward (blue) shifts in the pitch of auditory feedback. No significant difference was found between the effects of upward and downward shifts ($p=0.16$, 1-tailed t-test), and the mean changes in both groups (dashed red and blue lines) were not significantly different from zero ($p>0.12$ in both cases, 1-tailed t-tests). The red arrow indicates the value of the example shown in b. Plotting conventions as in Figure 3b in the main text.

Fundamental frequency does not predict learning across harmonic features

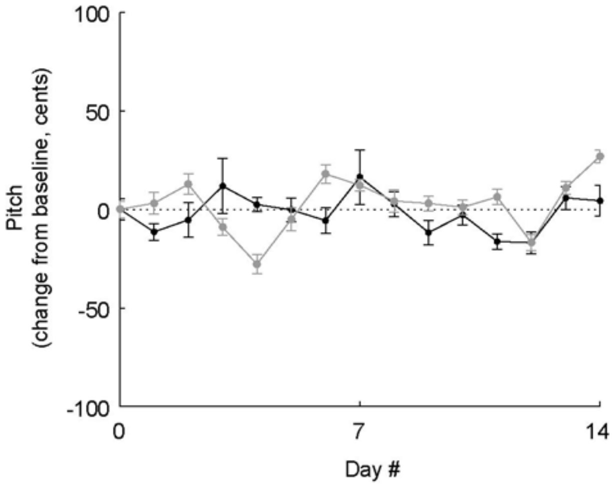


<u>Supplementary Figure 4:</u> Adaptive pitch change as a function of fundamental frequency. For each spectral frame, we plot the mean fundamental frequency in the baseline condition against the adaptive change in pitch (i.e. change in the direction opposite the imposed pitch shift). Symbol shapes identify individual birds as in Figure 3a in the main text. Data from upward and downward shifts in the pitch of auditory feedback (+/- 100 cents) are plotted in blue and red, respectively. There was no significant correlation between fundamental frequency and adaptive pitch change when data from upward and downward shifts were pooled (dashed black line, p=0.84) or when data from upward and downward shifts were considered separately (p=0.22 and p=0.96, respectively).



Supplementary Figure 5: Gradual shifts in feedback pitch drive large changes in the pitch of song. In three birds the pitch of auditory feedback was shifted gradually (by 35 cents every 6 days, as indicated by red and blue lines labeled to indicate the imposed shift as a function of time). Two birds experienced a sequence of upward shifts (filled and unfilled blue symbols) and one bird experienced a sequence of downward shifts (red symbols). Other plotting conventions are as in Figure 3a in the main text.

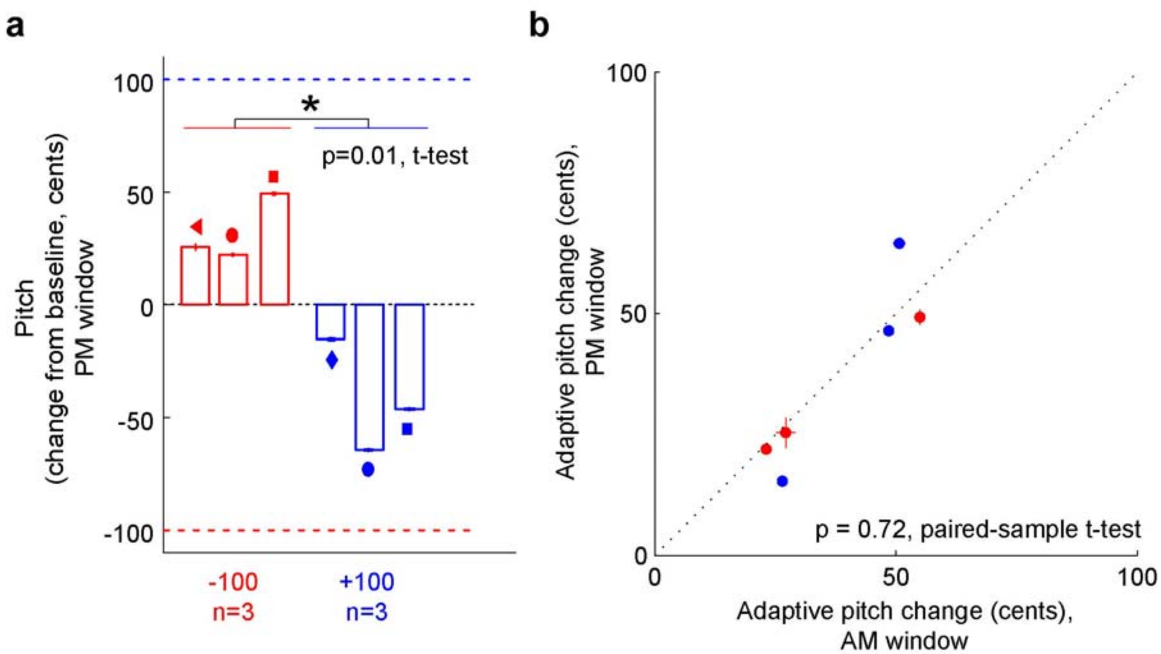
Time course data for 0-shift control birds



<u>Supplementary Figure 6:</u> Stability of song in the absence of pitch shift. Data from two control birds who wore headphones but were not subjected to shifts in the pitch of auditory feedback (plotted in black and gray for visual clarity). Other plotting conventions as in Figure 3a in the main text.

Adaptive pitch learning in evening song

In the results presented in the main text, only songs recorded in the late morning (between 10 am and noon) were analyzed. This sampling technique leaves open the question of whether evening song is similarly adaptable. This question is especially important given that prior work has shown significant differences between morning and evening song in young birds, and further demonstrated that the size of this difference predicts the final similarity to the song model (Deregnaucort et al. 2005). We addressed this issue by repeating the analysis shown in Figure 3b in the main text on song data recorded in the evening (between 6pm and 8pm). As shown in Supplementary Figure 7a below, the pitch of evening song changed in the adaptive direction (i.e. opposite the imposed pitch shift) in all 6 experiments. We then compared the magnitude of learning assayed in the evening window with that quantified in the original morning window on an experiment-by-experiment basis. As shown in Supplementary Figure 7b below, there was no significant difference between adaptive pitch changes during the two windows (p=0.72, paired-sample t-test). These results indicate that the manuscript's key finding is obtained independently of whether morning or evening song is used for data analysis.



<u>Supplementary Figure 7:</u> Comparison of adaptive song changes when measured in the morning and evening. **a**, Mean +/- S.E. changes in the pitch of song during shift days 12-14 when pitch is measured in a window from 6:00-8:00 pm, rather than the 10:00 am-noon window used in the main analysis. All other plotting conventions as in Figure 3b in the main text. **b**, Comparison of the magnitude of adaptive pitch changes (mean +/- S.E.) when measured in the morning and evening. Adaptive changes (i.e. changes in the direction opposite the imposed shift in auditory feedback) were not significantly different when measured in the morning or evening windows (p=0.72, paired-sample t-test).

Deregnaucourt S, Mitra PP, Feher O, Maul KK, Lints TJ, Tchernichovski O (2004) Song development: in search of the error-signal. Ann N Y Acad Sci 1016:364-376.