

LETTERS

Performance variability enables adaptive plasticity of 'crystallized' adult birdsong

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Significant trial-by-trial variation persists even in the most practiced skills. One prevalent view is that such variation is simply 'noise' that the nervous system is unable to control or that remains below threshold for behavioural relevance^{1–3}. An alternative hypothesis is that such variation enables trial-and-error learning, in which the motor system generates variation and differentially retains behaviours that give rise to better outcomes. Here we test the latter possibility for adult bengalese finch song. Adult birdsong is a complex, learned motor skill that is produced in a highly stereotyped fashion from one rendition to the next^{4,5}. Nevertheless, there is subtle trial-by-trial variation even in stable, 'crystallized' adult song^{6–8}. We used a computerized system to monitor small natural variations in the pitch of targeted song elements and deliver real-time auditory disruption to a subset of those variations. Birds rapidly shifted the pitch of their vocalizations in an adaptive fashion to avoid disruption. These vocal changes were precisely restricted to the targeted features of song. Hence, birds were able to learn effectively by associating small variations in their vocal behaviour with differential outcomes. Such a process could help to maintain stable, learned song despite changes to the vocal control system arising from ageing or injury. More generally, our results suggest that residual variability in well learned skills is not entirely noise but rather reflects meaningful motor exploration that can support continuous learning and optimization of performance.

Birdsong, like speech, is a learned motor skill that requires exceptionally precise and rapid control of vocal musculature; for both song and speech, modulation in acoustic structure occurs on a timescale of milliseconds^{4,9}. This fine level of vocal control is learned in a process that depends crucially on auditory feedback. In many songbird species the learned song 'crystallizes' into a stable form after reaching adulthood⁴. However, disruption of feedback can cause a gradual deterioration in song, suggesting the continued importance of feedback for monitoring and maintaining performance^{10–14}.

Prior modelling indicates that song learning could proceed through trial and error, in which variable songs are produced ('motor exploration') followed by selective reinforcement of better versus worse variants^{15–18}. Consistent with the possibility that song variation reflects motor exploration, experimental data indicate that a component of variability in both juvenile and adult song is centrally controlled^{7,8,19–22}. Despite these observations, it remains unknown whether birds can modify their songs by a trial-and-error process in which they associate variations in vocal behaviour with better versus worse outcomes.

We directly tested this possibility in adult bengalese finches by using a computerized system to continuously monitor song and provide online disruptive feedback for a targeted range of vocalizations. A normal song consists of a stereotyped sequence of discrete

'syllables' (Fig. 1a). We focused our experiments on the pitch, or fundamental frequency, of individual syllables, because fundamental frequency is learned, precisely controlled and readily quantifiable^{5,7,8}. The coefficient of variation of fundamental frequency for syllables in our study was 1.5% (median; range 1.2–2%). This only slightly exceeds the variation present across renditions of notes by trained vocalists and is much less than the variation across repeated phonemes for normal speech^{23,24}. Moreover, this variation is of the same order as frequency discrimination thresholds for birds and humans²⁵. Hence, variation in adult bengalese finch song is quite subtle, commensurate with that of other well-practiced skills.

An experiment to assess whether this variability can be used by the nervous system is illustrated in Fig. 1. We targeted fundamental frequency of syllable 'a' (Fig. 1a) for modification. Initially, fundamental frequency was tightly controlled with only ~1% variation (Fig. 1c; fundamental frequency = $2,281 \pm 27$ Hz). We used short bursts of white noise as a negative reinforcer for a subset of syllable variants, as white noise disrupts normal feedback and causes a gradual, non-specific deterioration of song when chronically used to disrupt feedback (see Methods). Here, rather than indiscriminately disrupting feedback, we delivered white noise in a manner contingent on acoustic features of the targeted syllable. Higher pitched renditions received a short latency burst of white noise ('hit'), whereas lower pitched versions received normal feedback ('escape') (Fig. 1b). Unlike natural feedback, which potentially provides fine-grained information about amplitude, pitch and other features of song, our experimentally controlled feedback provided a binary signal for each rendition of the target syllable, either 'bad' (white noise burst) or 'good' (escape), without further detail about the aspects of song that triggered feedback. We then asked whether birds could detect the externally imposed contingency that led to disruption of feedback and alter their songs to escape it.

This form of behaviourally contingent reinforcement drove rapid, adaptive changes to targeted features of adult song. For the experiment of Fig. 1, the fundamental frequency of the targeted syllable decreased significantly over the first three days of reinforcement (Fig. 1d; fundamental frequency reduced by 1.8 times the standard deviation of the baseline distribution; $P < 0.0001$). Correspondingly, the hit rate dropped from 91% (baseline) to 17%. By establishing the appropriate contingency, the same reinforcement scheme could direct either increases or decreases in pitch (Fig. 1e). When white noise was differentially delivered at higher frequencies, the fundamental frequency always shifted lower; when white noise bursts were delivered at lower frequencies, the fundamental frequency shifted higher (Fig. 1f). Hence, the applied contingency determined the direction of behavioural change ($P < 0.01$; sign-test for 7 of 7 cases shifting in the direction of the applied contingency). In all cases, changes in fundamental frequency led to a large reduction in hit rate,

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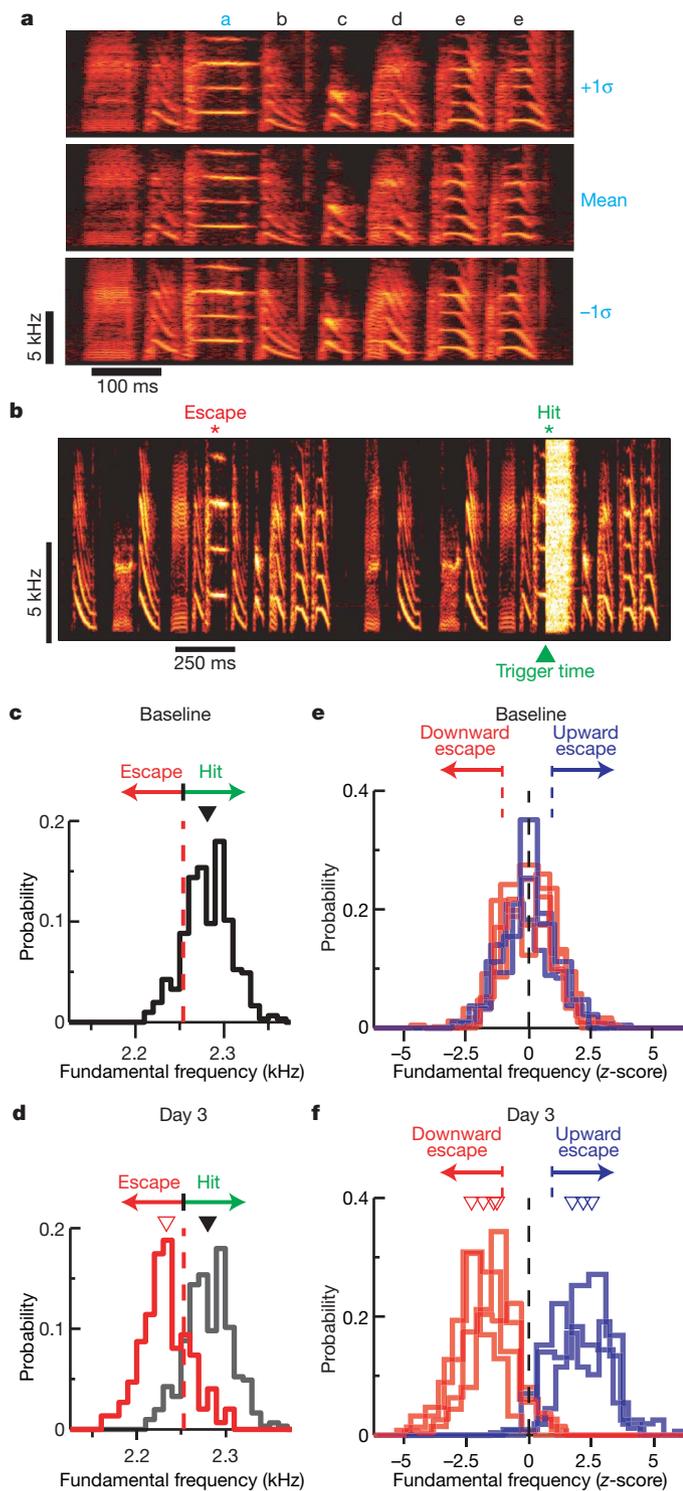


Figure 1 | Differential reinforcement can adaptively alter features of adult song. **a**, Syllables (a, b, c, d, e, e) are normally produced with little variation. Three songs are shown for which the fundamental frequency of ‘a’ spanned 2 standard deviations of the baseline distribution (Supplementary Recording 1 contains corresponding audio files). **b**, White noise bursts (‘hits’) were targeted at higher pitched versions of ‘a’. **c**, Baseline fundamental frequency distribution for ‘a’, showing overall mean (triangle) and mean for escapes (line). **d**, Fundamental frequency distribution after 3 days white noise. **e, f**, Baseline (**e**) and day 3 (**f**) distributions for seven experiments in which white noise directed either downward (red) or upward (blue) shifts in fundamental frequency. Fundamental frequency is expressed in units of the standard deviation of the baseline distribution (z-score, see Methods).

with the median decreasing from 91% to 21% by the third day. These data provide the first evidence that auditory reinforcement signals can direct specific, adaptive changes to adult song. As a corollary, they demonstrate that birds were able to effectively associate small natural variations in vocal production with differential outcomes.

Changes elicited by differential reinforcement occurred rapidly. A detailed examination of one experiment reveals that fundamental frequency approached the asymptotic range within one day (Fig. 2a). Similarly, for six of seven birds, significant adaptive changes to fundamental frequency occurred within 7 h (Fig. 2b, ‘morning’) and for all birds significant changes occurred within the first day (Fig. 2b, ‘evening’). The median number of syllables sung within the first half-day and full day were 605 and 1,179, respectively. By day 3, fundamental frequency stabilized at nearly asymptotic values (Fig. 2b).

The induced changes in fundamental frequency recovered rapidly. After at least 3 days of reinforcement, contingent white noise bursts were terminated. In every case, the fundamental frequency reverted towards its original range (Fig. 2c). Hence, the nervous system retains a representation of the initial song and both the capacity and impetus to return song towards its original structure in the absence of externally imposed drive.

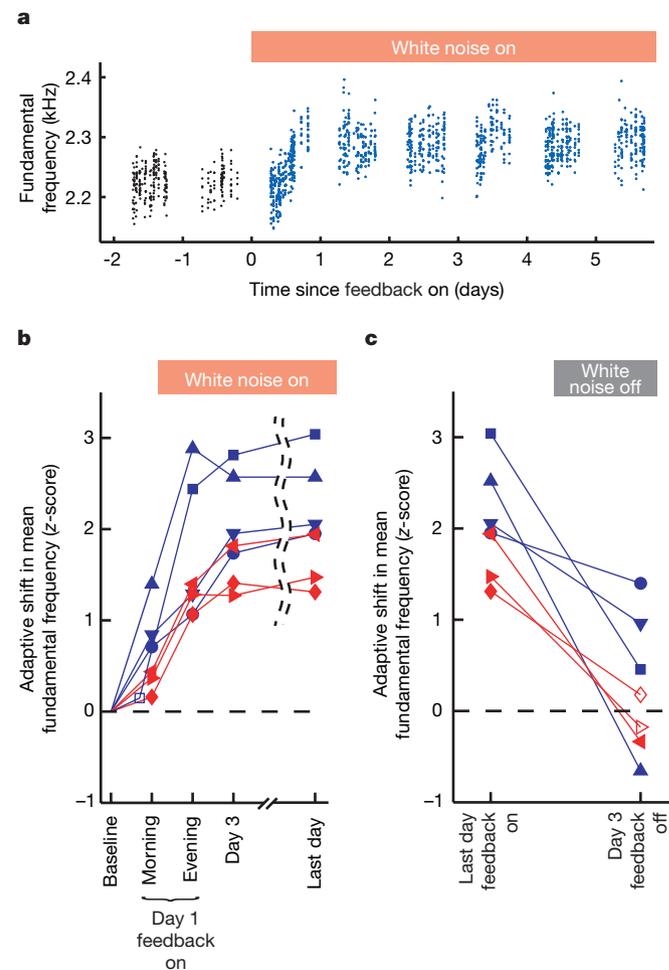


Figure 2 | Adaptive shifts in fundamental frequency occur rapidly and recover. **a**, Fundamental frequency of targeted syllables for one adult bird (age 334 days). Fundamental frequency progressively increased during the first day. **b**, Upward (blue) and downward (red) shifts in fundamental frequency. Filled symbols indicate significance ($P < 0.05$). After 3 days, fundamental frequency changed little, indicating that shifts had reached near asymptotic values. **c**, Fundamental frequency for last day with white noise feedback on (‘Last day, feedback on’) and third day following termination of feedback (‘Day 3, feedback off’).

In principle, birds could escape white noise equally well by altering the fundamental frequency of only the targeted syllable or of larger segments of song. Nevertheless, we consistently found that changes to fundamental frequency were restricted exclusively to targeted syllables (Fig. 3a, b, red symbols). Other syllables, even when they occurred within a few tens of milliseconds, did not change (Fig. 3a, b, blue symbols). Furthermore, the changes were specific to fundamental frequency and did not affect other features such as duration, volume and spectral entropy (Fig. 3c). Hence, even though the experimentally imposed contingency between performance and feedback was not revealed to the bird in any direct sense, the nervous system was able to detect and respond precisely to that contingency.

This specificity indicates an impressive capacity of the nervous system to modify discrete features of song independently. This is appropriate for vocal learning, where birds match their song to rapidly varying features of an acoustic model. If modification of one song feature generalized to cause modification of others, learning might still proceed, but such interference would probably slow its progress²⁶.

In theory, reinforcement signals can drive learning even at long latencies to the actions that precipitate them¹⁵. For complex motor

skills, however, the nervous system might detect the contingency more effectively at shorter delays. We tested the importance of delay by varying the time between measurement of fundamental frequency

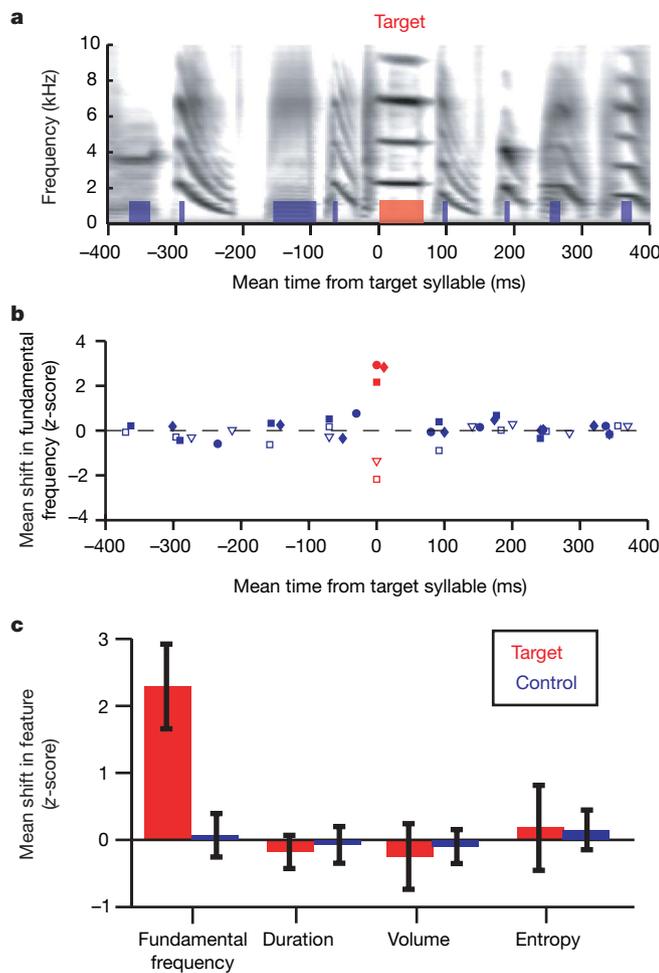


Figure 3 | Changes are restricted to targeted features of song. **a**, Spectrogram illustrating analysed features for target (red bar) and control (blue bars) syllables of an individual experiment. **b**, Mean changes in fundamental frequency for target (red) and control (blue) syllables. Squares represent two experiments for song illustrated in **a**. Data from 3 additional birds (circles, triangles, diamonds) are shown without corresponding spectrograms. Filled and open symbols indicate experiments with upward and downward shifts in fundamental frequency, respectively. **c**, Spectral characteristics other than fundamental frequency were not altered for either target (red) or control (blue) syllables. Bars indicate mean \pm standard deviation.

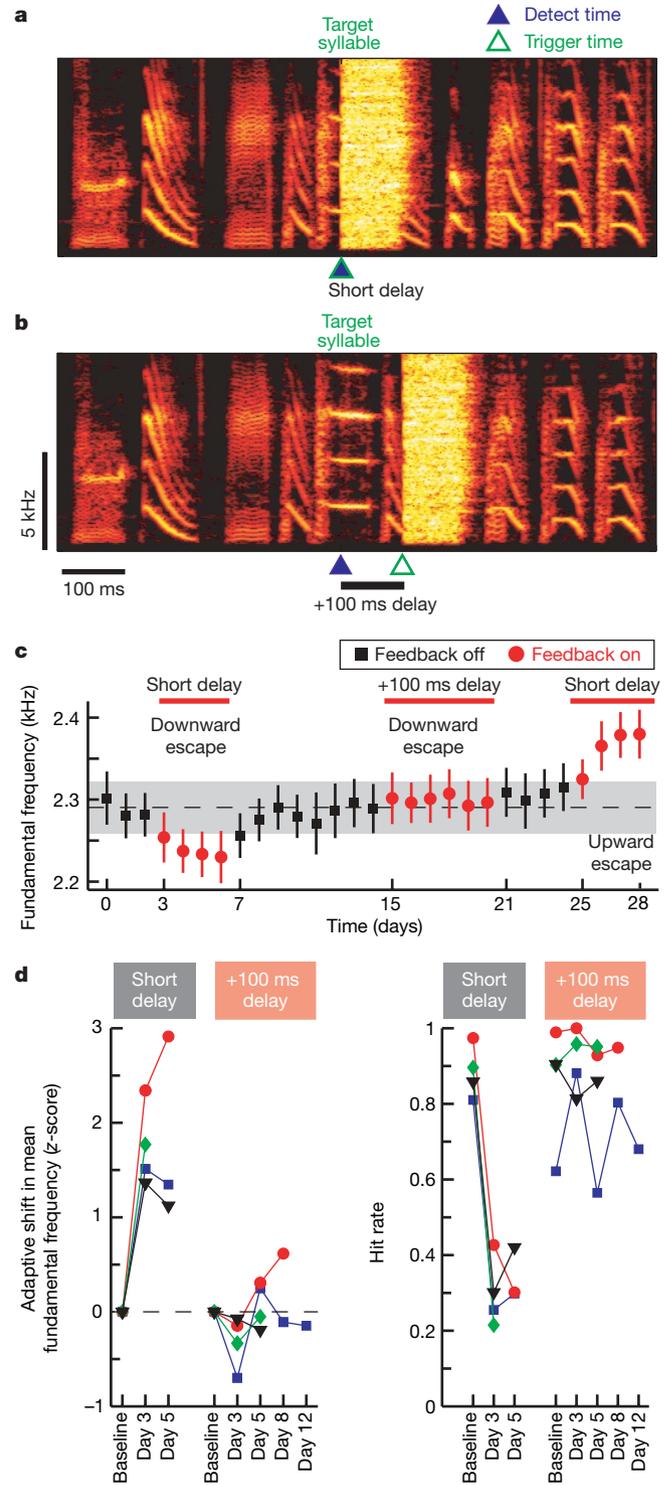


Figure 4 | Delayed feedback prevents adaptive pitch shifts. **a**, **b**, Spectrograms illustrating short delay (**a**) and +100 ms delay (**b**) feedback for one bird. **c**, Fundamental frequency (mean \pm standard deviation) for this bird. Initially, short delay feedback directed a downward shift in fundamental frequency. After recovery, the same contingency with delayed feedback was ineffective. Subsequently, an upward shift was driven using a new short delay contingency. Shading indicates baseline ± 1 standard deviation. **d**, Summary of shifts in fundamental frequency and hit rate. Symbols indicate 4 birds subjected to both short delay and +100 ms delay reinforcement. Shifts were prevented when feedback was delayed.

and delivery of feedback. White noise typically was delivered within 16–30 ms. In this case, white noise overlapped with the targeted syllable (Fig. 4a, ‘short delay’). For some experiments, white noise was delayed by an extra 100 ms. In this case, white noise started after completion of the targeted syllable (Fig. 4b, ‘+100 ms delay’). Syllables targeted at short delay rapidly shifted and reached asymptotic values after roughly 3–4 days. In contrast, the same syllables, when targeted with delayed feedback, exhibited no directed change to fundamental frequency even over longer periods (Fig. 4c, d). Hence, even small delays profoundly attenuated adaptive responses. Likewise, in the +100 ms condition, there were no systematic changes to syllables following the targeted syllable, for which white noise was delivered in a non-contingent manner (see Supplementary Fig. 3). Hence, rapid changes to fundamental frequency required both that white noise was contingent on the structure of a syllable and that it occur at short delay. These data suggest that the vocal control system may take advantage of normally predictable timing between premotor activity and resultant sensory consequences; normally, activity of premotor neurons influences song and resultant feedback at latencies < 70 ms (ref. 27). Therefore, the nervous system may not readily detect reinforcement contingencies when feedback is delayed beyond this range. Although such timing constraints are not necessary for reinforcement learning, they potentially enable more efficient learning and may be a general feature of sensorimotor systems where there is predictable delay between action and consequence²⁸.

Our results indicate that small natural variations present in adult song can enable adaptive modification of targeted features. These changes exceed the frequency discrimination threshold for birds²⁵. Moreover, the reversion of fundamental frequency after termination of feedback (Fig. 2c) indicates the nervous system is sensitive to the discrepancy from the original song. Nevertheless, our reinforcement scheme constrained shifts in fundamental frequency to be similar in magnitude to the initial range of variation (see Supplementary Fig. 2). This raises the question of whether changes in adult song are restricted to subtle fine-tuning, or whether more dramatic remodeling is possible. We tested this by progressively altering the reinforcement contingency so that continued escape from white noise would require progressively larger shifts in fundamental frequency (Fig. 5). We were able to incrementally drive large changes in fundamental frequency, such that syllables eventually were produced in a range completely non-overlapping with the baseline range (Supplementary Movie 1 demonstrates the salience of these changes). This finding implies that following each incremental shift in fundamental frequency a new range of behaviourally relevant variation was established that enabled differential reinforcement of more extreme vocalizations. The range of variation remained relatively constant over time (for example, see Figs 1, 4, 5). Hence, as learning progresses, current performance is continuously surrounded by a ‘halo’ of variation that enables continuous adaptive modification.

In summary, our results demonstrate that binary feedback signals can drive rapid plasticity of normally stable adult song. In contrast to previous studies in which disruption of feedback led to a deterioration of song^{10–14}, we show that differentially delivered feedback can direct precise, adaptive changes to adult song. The changes are precise in that they are restricted to targeted features. They are adaptive in that birds alter those features systematically to escape disrupted feedback. These data provide empirical support for models suggesting that song might normally be shaped by reinforcement signals^{16–18}. More generally, they indicate that the trial-and-error process of reinforcement learning can efficiently guide adaptive modification of highly complex and tightly controlled motor skills.

The adaptive plasticity reported here also demonstrates that birds can accurately associate small variations in fundamental frequency with resultant consequences. In principle, variation could be monitored by sensory feedback (auditory or proprioceptive). Alternatively, if variation is centrally generated, it could be monitored

internally by an efference copy of premotor activity¹⁷. Consistent with this possibility, studies in both birds and mammals indicate that some movement variability derives from central neural activity rather than the periphery^{7,8,29,30}, suggesting that variability may be actively generated for motor exploration. Regardless of mechanism, our data indicate that natural variations present in crystallized adult song are not simply noise but rather can be exploited for trial-and-error

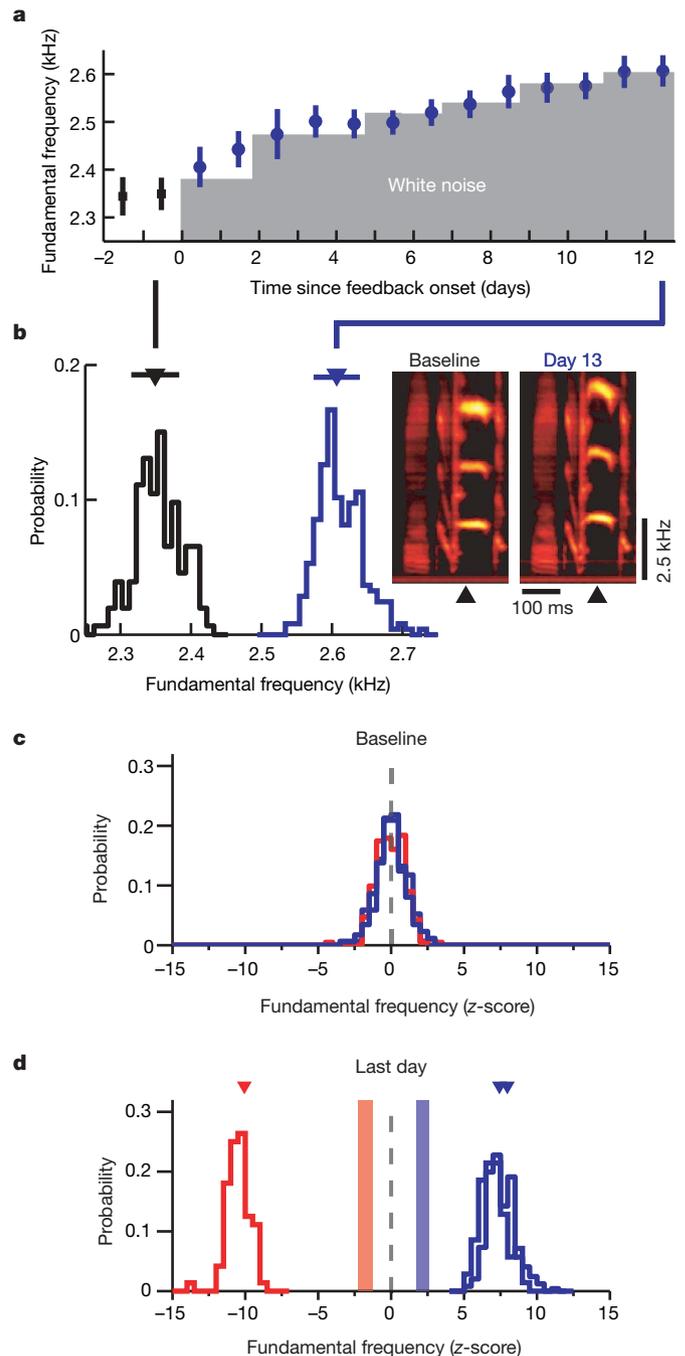


Figure 5 | Incremental adjustment of threshold drives large pitch changes. **a**, Changes to fundamental frequency for one experiment. Points indicate mean (\pm standard deviation) on each day. Shading indicates threshold for escapes. **b**, Fundamental frequency distributions for baseline (black) and day 13 (blue). Spectrograms show the average syllable for corresponding days. **c**, **d**, Baseline (**c**) and final (**d**) fundamental frequency distributions for three experiments with thresholds incrementally adjusted upward (blue) or downward (red). Over 11–14 days, mean fundamental frequency (triangles) shifted by 7.0–10.5 standard deviations of the baseline distribution (226–376 Hz). For comparison, vertical shaded regions show the range of mean changes driven with a fixed reinforcement contingency.

learning. We suggest that in other systems as well, subtle variation in performance may reflect continued experimentation to optimize behaviour, rather than noise that the nervous system is unable to control, or that is below threshold for behavioural relevance.

METHODS SUMMARY

Subjects. Eleven adult (age 112–346 days) male bengalese finches (*Lonchura striata var. domestica*) were used. All procedures were approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

Song recording and behaviourally contingent reinforcement. Birds were housed individually in sound isolation chambers throughout the experiments. Custom LabView software (National Instruments) was used for song recording and online detection of target syllables. We tuned spectral templates to differentially detect and trigger reinforcement of higher or lower pitched syllables, thereby establishing a clear direction in which pitch would need to be shifted to reduce disruptive feedback.

Hit rate and syllable structure calculations. Hit rate was defined as the percentage of times the target syllable matched the established criterion for white noise playback out of the total number of target syllables sung. Fundamental frequency was calculated from the frequency bins surrounding the harmonic peaks in the power spectrum of the target syllable. Volume of individual syllables was normalized by mean volume of the entire song. Duration and spectral entropy (from 0.5–10 kHz) were calculated as previously described²⁷. Significance was determined using two-tailed *t*-tests. During baseline periods, hit rate was calculated in the absence of white noise playback. During the period of contingent reinforcement, we randomly selected a subset of songs as ‘catch’ trials for which white noise playbacks were suppressed (10–20%). The values reported for hit rate, fundamental frequency and other syllable parameters were derived from these catch trials.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions E.C.T. performed the experiments and analysis; E.C.T. and M.S.B. designed the experiments and wrote the manuscript.

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METHODS

Song recording and behaviourally contingent reinforcement. Birds were housed individually in sound isolation chambers (Acoustic Systems) with a 14/10 h light/dark cycle throughout the experiments. Custom LabView software (National Instruments) was used for song recording and online detection of target syllables. Before initiation of contingent reinforcement, songs were recorded for several days to measure baseline statistics on fundamental frequency of targeted syllables and develop spectral templates to detect syllables in real-time and trigger playback of white noise contingent on syllable spectral structure. For 2 birds a diurnal trend in pitch was apparent during baseline, reminiscent of that in juvenile zebra finches⁵ (for example, Fig. 2a), but did not correlate with observed plasticity. Templates were constructed from the power spectrum of short segments (8 ms) of the target syllable. By varying the exemplar syllables from which templates were constructed, we tuned templates to differentially detect and trigger reinforcement of higher or lower pitched syllables. During the baseline period (with no white noise playback), we monitored the distribution of fundamental frequency for 'hits' and 'escapes' to determine empirically the actual feedback contingency that was applied in each experiment. For most experiments, 'escapes' were present only at the upper (or lower) end of the natural range of variation for fundamental frequency, establishing a clear direction in which pitch would need to be shifted to reduce disruptive feedback. For these experiments, an adaptive response was defined as a shift in mean pitch towards the end of the baseline distribution that contained escapes. See Supplementary Information for additional details on the timing and specificity of contingent reinforcement. For two experiments, escapes were present at both the upper and lower ends of the baseline distribution. In both of these cases, birds shifted the pitch of the targeted syllables upwards and reduced the hit rate. However, in these cases, the direction of adaptive response (increase versus decrease in pitch) was not clearly defined by the initial feedback contingency, and therefore these experiments are not included in analyses documenting that changes to targeted features of song can be specifically directed by predetermined feedback contingencies (for example, Fig. 1).

White noise bursts were used to differentially reinforce variations within the normal range of vocalizations. The duration of white noise bursts was long enough to overlap with the remainder of the targeted syllable (80–200 ms). We chose white noise bursts as our stimulus for differential reinforcement for two reasons. First, the superposition of white noise on normal feedback disrupts the sound that the bird experiences, and previous experiments have shown that chronic (non-contingent) disruption of feedback in this fashion results in a gradual and non-specific deterioration of song^{14,31,32}. Second, perturbation of

feedback with similar stimuli for both birds and humans can cause acute disruptions of ongoing vocalizations²⁷. We confirmed for each of our experiments that white noise bursts were behaviourally salient in that they caused a significant increase in the probability of song stoppages during the first day of exposure. These acute effects typically habituated over the course of extended exposure. Nevertheless, they suggest that white noise stimuli superimposed on song are readily detected by the nervous system. Our finding that birds consistently alter their songs to avoid white noise bursts indicates that this form of feedback disruption indeed acts as an effective negative reinforcer.

Hit rate and fundamental frequency calculation. Hit rate was defined as the percentage of times the target syllable matched the established criterion for white noise playback out of the total number of target syllables the bird sang. During baseline periods, hit rate was calculated in the absence of white noise playback. During the period of contingent reinforcement, we additionally suppressed white noise playbacks for a randomly selected subset of songs (10–20%). These catch trials allowed us to monitor the progression of changes to pitch (and hit rate) for songs that were produced with entirely normal feedback, avoiding any potential complications due to online contributions of feedback to song structure. The values reported for fundamental frequency and hit rate are derived from these catch trials.

Online evaluation of fundamental frequency for purposes of contingent reinforcement relied on short (8 ms) segments of targeted syllables and a rapid template-matching algorithm (see above) in order to minimize the latency between production of song features that met criterion and the delivery of white noise. For analysis of spectral characteristics of targeted syllables, longer segments (16–64 ms) of the syllable waveform were used. Each segment was Fourier transformed and the fundamental frequency was calculated from the average of the five frequency bins neighbouring the peak weighted by the corresponding spectral power in each bin. Volume of individual syllables was normalized by mean volume of the entire song. Duration and spectral entropy (from 0.5–10 kHz) were calculated as previously described²⁷. To summarize effects across syllables, we expressed changes in measured features as *z*-scores, computed as the difference between the means of the experimentally shifted and baseline distributions, divided by the standard deviation of the baseline distribution. Significance was determined using two-tailed *t*-tests.

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