Supporting Information

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SI Materials and Methods

Subjects. Subjects were adult male zebra finches (*Taeniopygia guttata*). Their care and treatment were reviewed and approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco.

Surgery. Under Equithesin anesthesia (1), bilateral lesions of Area X were made in birds 104–118 d posthatch by pressure injections of 1% ibotenic acid (0.1–0.2 μL) (PMI-100; Dagan). Two to four injections were stereotaxically targeted on each side over 1–2 d. A subset of birds was deafened by bilateral cochlear removal 0–2 d following the last injection (2). Lesions of Area X were evaluated in brain sections labeled with an antibody to substance P (Accurate Chemical and Scientific) (3). To estimate lesion size, the remaining volume of Area X (average of right and left) was expressed as a percentage of the mean volume of intact Area X from six control birds. Using brain sections labeled with an antibody to calcitonin gene-related peptide (CGRP; Chemicon International) (4), we confirmed that LMAN (lateral magnocellular nucleus of the nidopallium) was spared by measuring its volume and visually inspecting its axonal projections to the RA (robust nucleus of the arcopallium). For all of the lesion birds, the percentage of Area X that was removed bilaterally ranged from 75–100%. Sham lesions were made by injecting vehicle (0.2 M phosphate buffer) or a small volume of ibotenic acid into Area X, or by making small electrolytic lesions above LMAN [data from two sham-lesioned birds were collected previously (5), and are replotted in Fig. 2*C* for comparison]. Area X volumes were confirmed to be > 90% of intact Area X. A third, separate group of birds was deafened as above for comparison with intact, lesion-deaf, and lesion-hearing birds.

Song Analysis. To examine changes in song structure, we compared the spectral and temporal structure of songs recorded 0–16 d before and 8 wk (±1 wk) after experimental manipulations (i.e., Area X lesions and/or deafening). At each time point, > 50 bouts of song were recorded from birds isolated in sound attenuating boxes ("undirected" songs).

Spectral change. To assess changes in song spectral structure, we randomly chose 5 and 10 motifs from pre- and postoperative songs, respectively, and made pair-wise comparisons between all possible pre- versus postoperative motif combinations. A motif was defined as a stereotyped sequence of syllables that occurred most frequently in pre- and in postoperative songs, respectively. Spectral similarity of song motifs was quantified using the percent similarity measure of Sound Analysis Pro (v1.04) (6). This measure quantifies the percentage of a postoperative motif that matches a preoperative motif using six acoustic features (pitch, frequency modulation, amplitude modulation, Wiener entropy, pitch goodness, and mean frequency), providing a measure of similarity of the overall acoustic structure of motifs. Default values were used for all parameters except the time warping tolerance, which was set to 20%. Raw similarity scores (S_{raw}, ranging 0–100) were normalized to the average score of motif pairs from unrelated normal birds (S_{min}) using the following equation:

$$\text{Normalized similarity score} = S_{raw} - S_{min} / S_{max} - S_{min}.$$

S_{min} (= 41.2) was calculated from 90 comparisons of randomly chosen motif pairs from 10 unrelated birds. S_{max}, the similarity of identical motifs, is 100 by definition. Thus, a normalized similarity score of zero indicates that pre- and postoperative motifs from the same bird are as different as two motifs from unrelated birds, and a normalized similarity score of 1 indicates that the motifs are identical.

Temporal change. To assess changes in song temporal structure, we compared the log-amplitude envelopes of 5 preoperative motifs and 10 postoperative bouts. A song bout was defined as a period of singing separated by > 2 s of silence. We searched postoperative bouts for amplitude patterns that provided the closest temporal match to the preoperative motif by calculating a cross-correlation function and taking the maximum value of the function. Because some songs speeded up over time, we allowed for proportional changes in the temporal pattern of songs (±20%) when searching for the maximum correlation. Maximum cross-correlation functions were normalized in the same manner as above; 0 indicates no more preservation of temporal pattern than random songs and 1 indicates complete preservation of temporal pattern.

Song variability. To examine variability in song structure across renditions, we randomly chose 20-80 motifs from pre- and postoperative songs, and measured the fundamental frequency (FF) of syllables with clear harmonic structure as described previously (5). Since many syllables show slow fluctuations of FF over time during a day, we normalized FF variability by the diurnal fluctuations as follows: for each syllable rendition, the raw FF value was normalized by the mean FF computed across all syllable renditions in a one hour (±30 min) window around the target syllable, and the percent difference from the local mean FF was obtained. Variability in FF was expressed as the RMS of percent differences obtained from all syllable renditions.

Neural Recording and Neural Data Analysis. Recordings of LMAN activity were performed as described previously (1). Briefly, extracellular signals were recorded using tungsten electrodes in a lightweight microdrive secured to the skull. LMAN was targeted stereotaxically or with the aid of antidromic stimulation of RA-projecting LMAN neurons through a bipolar stimulating electrode placed in RA. The majority of recordings were made when males sang alone; in a subset of experiments, LMAN activity was recorded when birds produced songs directed at females (courtship song) (7). At the conclusion of experiments in each bird, small electrolytic lesions were made at previously recorded sites. Electrode tracks and lesions were localized in CGRP-stained brain sections. Locations of recording sites were confirmed by their position relative to the depth of marker lesions.

Analysis of LMAN activity was performed as described previously (7). Action potentials were sorted off-line using Bayesian spike-sorting techniques, and sites were identified as a single unit or a small cluster of units. Isolation of single units was verified by visual inspection of the waveforms and by the presence of a refractory period in the interspike interval (ISI) histogram (refractory period ≤ 0.7 ms; < 1% ISI violations for each single unit). To characterize baseline, nonsinging activity, we analyzed spike trains that were recorded during quiet periods in which no sounds were produced; such periods were required to precede song initiation by at least 3 s and to follow song termination by at least 3 s (1). To characterize singing-related LMAN activity, songs and spike trains were linearly time-warped using a template for the amplitude envelope of the bird's motif (7). Spike bursts were defined as groups of two

or more action potentials separated by ≤ 5 ms, and we quantified the fraction of all spikes that occurred as part of bursts (burst fraction).

Motif-aligned firing-rate histograms were computed using a sliding window (20 ms), and modulation of the histograms was quantified as the coefficient of variation (CV). To estimate firing-rate modulations in individual trials, the instantaneous firing rate during each motif rendition was estimated by smoothing the spike train with a Gaussian filter (SD = 5 ms), and the CVs of the firing rate functions were averaged across all trials. To quantify trial-by-trial variability in LMAN activity, we computed the correlation coefficient (CC) between the instantaneous firing rate functions for all pairs of trials (7). CCs were also calculated for all pairs of spike trains after a random time shift was added, and the distribution of time-shifted correlations provided a zero-correlation baseline for comparison with our results (8). For motif-aligned firing-rate histograms and CCs of instantaneous firing rates, the results were similar across a range of filter widths (10–40 ms and 2–20 ms, respectively).

Statistics. Comparisons of song plasticity between different experimental groups were made using the analysis of variance and post hoc Fischer's Protected Least Significant Difference tests. Comparisons of LMAN firing statistics, including mean CCs, were made using the Mann–Whitney *U* test. CC distributions were compared between actual and time-shifted spike trains using the Kolmogorov–Smirnov test.

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8. Ölveczky BP, Andalman AS, Fee MS (2005) Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. *PLoS Biol* 3(5):e153.

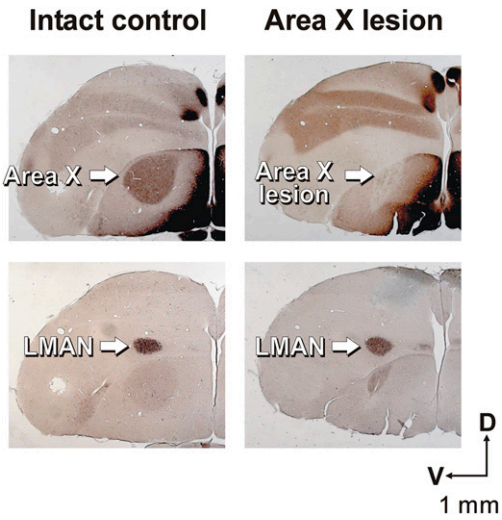

Fig. S1. Substance P-labeled (*Upper*) and CGRP-labeled (*Lower*) coronal brain sections through the left anterior forebrain. Area X and LMAN were labeled in an intact bird (*Left*). Area X was absent in a lesion bird, and LMAN was spared (*Right*).

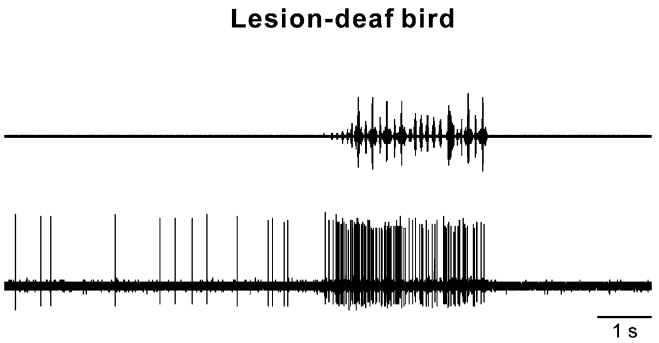

Fig. S2. Raw trace of singing-related activity of a single LMAN neuron in a lesion-deaf bird. LMAN firing (*Lower*) increased before the onset of song (*Upper*) and was depressed after song termination.

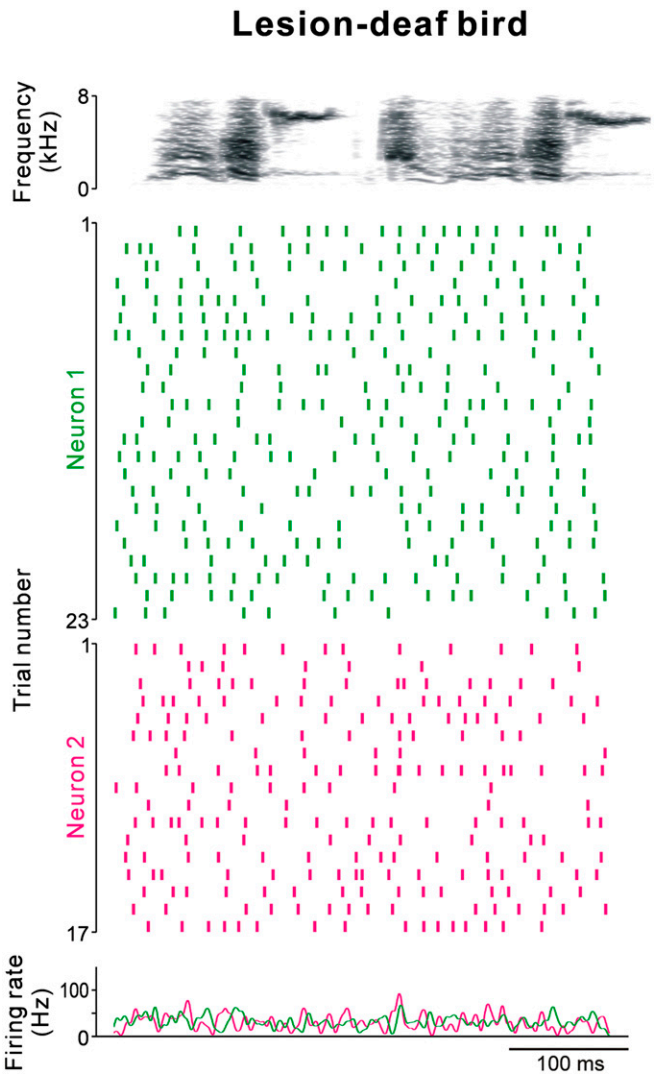

Fig. S3. Motif-aligned raster plots of singing-related activity (*Upper*) and corresponding firing rate histograms (*Lower*) during undirected singing of two LMAN neurons in a lesion-deaf bird. Firing rates were not strongly modulated during the motif, and neither neuron fired bursts of action potentials.

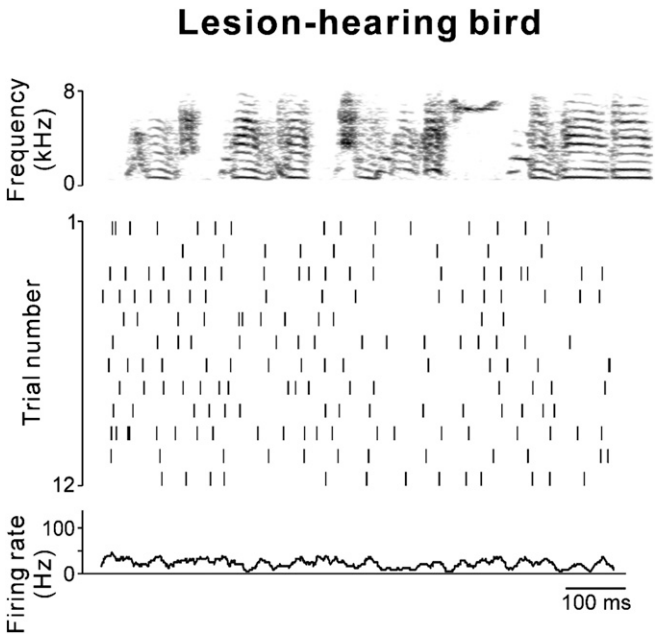

Fig. S4. Motif-aligned raster plot of LMAN activity during undirected singing in a lesion-hearing bird. Note the loss of bursts and song-locked firing patterns as in lesion-deaf birds.

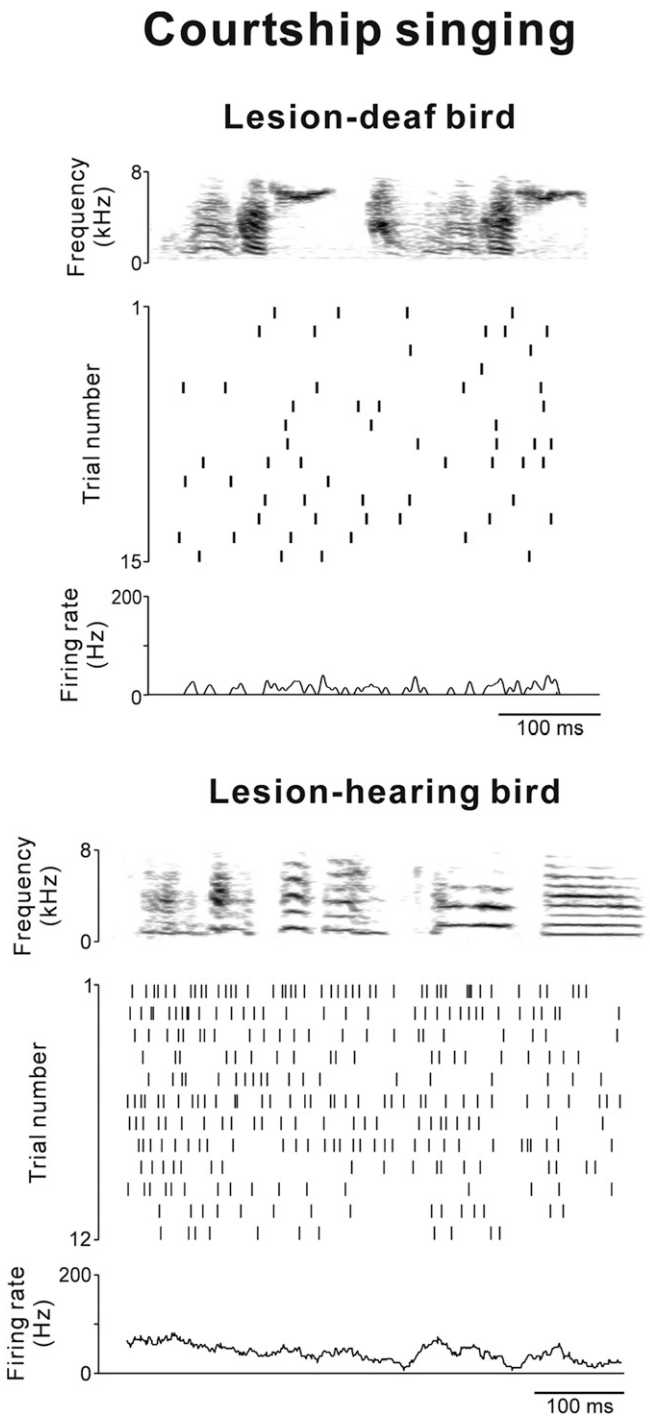

Fig. S5. Motif-aligned raster plots of LMAN activity during courtship singing in a lesion-deaf bird (*Upper*, same bird as in Fig. 5, *Right*) and a lesion-hearing bird (*Lower*). Note the absence of precise, song-locked firing patterns.