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COGNITIVE NEUROSCIENCE

Early onset of deafening-induced song deterioration and differential requirements of the pallial-basal ganglia vocal pathway

Haruhito Horita,[1,2] Kazuhiro Wada[1,3] and Erich D. Jarvis[1]
[1]Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710, USA
[2]School of Fundamental Science and Technology, Keio University, Yokohama, Japan
[3]Faculty of Advanced Life Science, Hokkaido University, Sapporo, Japan

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Abstract

Similar to humans, songbirds rely on auditory feedback to maintain the acoustic and sequence structure of adult learned vocalizations. When songbirds are deafened, the learned features of song, such as syllable structure and sequencing, eventually deteriorate. However, the time-course and initial phases of song deterioration have not been well studied, particularly in the most commonly studied songbird, the zebra finch. Here, we observed previously uncharacterized subtle but significant changes to learned song within a few days following deafening. Syllable structure became detectably noisier and silent intervals between song motifs increased. Although song motif sequences remained stable at 2 weeks, as previously reported, pronounced changes occurred in longer stretches of song bout sequences. These included deletions of syllables between song motifs, changes in the frequency at which specific chunks of song were produced and stuttering for birds that had some repetitions of syllables before deafening. Changes in syllable structure and song bout sequence occurred at different rates, indicating different mechanisms for their deterioration. The changes in syllable structure required an intact lateral part but not the medial part of the pallial-basal ganglia vocal pathway, whereas changes in the song bout sequence did not require lateral or medial portions of the pathway. These findings indicate that deafening-induced song changes in zebra finches can be detected rapidly after deafening, that acoustic and sequence changes can occur independently, and that, within this time period, the pallial-basal ganglia vocal pathway controls the acoustic structure changes but not the song bout sequence changes.

Introduction

Similar to humans, songbirds are one of the few groups that learn vocalizations (Marler, 1970; Doupe & Kuhl, 1999; Jarvis, 2004). Like other forms of learning, vocal learning requires the coordination of sensory and motor behavior by using auditory feedback to guide one's own vocalizations during a developmental phase of life. Auditory feedback also plays an important role in maintaining learned vocalizations in adults (Nordeen & Nordeen, 1992; Okanoya & Yamaguchi, 1997; Woolley & Rubel, 1997; Leonardo & Konishi, 1999; Lombardino & Nottebohm, 2000; Brainard & Doupe, 2001; Sakata & Brainard, 2006). In adult humans and songbirds, deafening causes learned vocalizations to deteriorate and the rate of deterioration is dependent upon age (Hammarberg *et al.*, 1980; Cowie *et al.*, 1982; Cowie & Douglas-Cowie, 1983; Ball *et al.*, 1990; Waldstein, 1990; Lane & Webster, 1991; Lombardino & Nottebohm, 2000; Brainard & Doupe, 2001), i.e. the older the individual the longer it takes for the vocalizations to deteriorate. For the most-studied songbird species, the zebra finch, a juvenile develops its song from highly variable vocalizations to the crystallized adult form by 90–100 days post-hatch (Immelmann, 1969). In old adults (> 1 year) deafening-induced changes can take several months, whereas in young adults (~90–130 days post-hatch) quantitative changes have been reported to occur by 4 weeks but changes in less than 4 weeks have been equivocal. Furthermore, the precise pattern and timing of deterioration remain unknown (Lombardino & Nottebohm, 2000; Brainard & Doupe, 2001).

The known deafening-induced vocal changes in adult zebra finches require an intact pallial-basal ganglia vocal pathway, also called an anterior vocal pathway (Fig. 1), which shares similarities with mammalian cortical-basal-ganglia-thalamic loops (Reiner *et al.*, 2004; Doupe *et al.*, 2005; Jarvis *et al.*, 2005). In this pathway, lesions to the pallial nucleus, the lateral magnocellular nucleus of the anterior nidopallium (LMAN), prevent deafening-induced changes in syllable structure and song motif sequence (Brainard & Doupe, 2000, 2001), and prevent modulation of song spectral structure (Kao *et al.*, 2005; Kao & Brainard, 2006). However, LMAN modulates the vocal motor output nucleus of the telencephalon, the robust nucleus of the

Correspondence: Dr K. Wada and Dr E. D. Jarvis, [1]Department of Neurobiology, as above.
E-mail: wada@sci.hokudai.ac.jp and jarvis@neuro.duke.edu

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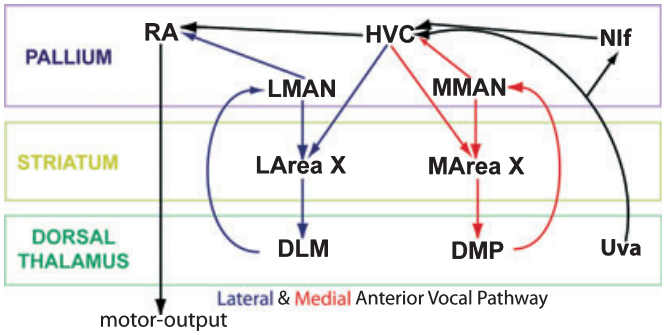

FIG. 1. Simplified schematic of the song pathway in songbirds. Both lateral and medial parts of the anterior vocal pathway are shown. Figure is modified from Jarvis *et al.* (1998). Connectivity is summarized in Jarvis *et al.* (1998) and Kubikova *et al.* (2007). DLM, dorsal lateral nucleus of the dorsomedial thalamus; DMP, dorsomedial nucleus of the posterior thalamus; LArea X, lateral Area X; MArea X, medial Area X; NIf, nucleus interface of the nidopallium; Uva, nucleus uvaeformis.

arcopallium (RA) (Fig. 1), and RA has been proposed to control syllable structure (Yu & Margoliash, 1996; Kao *et al.*, 2005; Kubikova *et al.*, 2007), whereas the medial magnocellular nucleus of the anterior nidopallium (MMAN) modulates nucleus HVC (HVC) (a letter-based name) and HVC has been proposed to control syllable sequences (Yu & Margoliash, 1996; Foster *et al.*, 1997; Kubikova *et al.*, 2007). MMAN lesion-induced changes in song sequencing were observed in adult birds but were larger in juvenile birds (Foster & Bottjer, 2001) and thus MMAN might play a role in song sequence. MMAN lesions also affect activity-dependent egr-1 gene expression in HVC (Kubikova *et al.*, 2007) and indirectly suggest that MMAN could be involved in song sequencing through HVC, as hypothesized in Kubikova *et al.* (2007). Based on these ideas, we hypothesized that lesions to LMAN in deafened birds would be expected to prevent changes in syllable structure, whereas lesions to MMAN would be expected to prevent changes in syllable sequence. Here, we tested this hypothesis by examining the acoustic structure and sequences of syllables in song bouts as opposed to only motifs, the time-course of deafening-induced changes and the possible requirements of the anterior vocal pathway for these changes. We found that significant quantitative changes occurred in syllable structure and song bout sequences rapidly following deafening in young adults and that only the syllable structural changes depended upon LMAN function, whereas the song bout sequences changes did not depend on either LMAN or MMAN. These findings suggest that LMAN via RA modulates syllable structure but song bout sequencing could be modulated independently of the vocal pallial-basal-ganglia loop.

Materials and methods

Animals

We used 29 young adult male zebra finches from our breeding colony, ranging from 92 to 138 days post-hatch. All animal procedures were approved by the Duke University Institutional Animal Care and Use Committee.

Definitions

We define 'song syllable structure' as the acoustic structure of a syllable, a 'song motif sequence' as the most frequently repeated sequence of syllables of a bird's song, commonly known as the song motif, and 'song bout sequence' as the sequence of all syllables in a song bout (inclusive of the motif syllables and syllables outside motifs such as introductory syllables and post-motif syllables). A song bout was defined as a continuous production of song followed by at least a 400 ms period of silence.

Behavioral analyses

Behavior

For 3–7 days before surgery, all birds were isolated and housed individually in sound-attenuation chambers (75 × 27.5 × 28.8 cm) (Tchernichovski *et al.*, 2000). The photoperiod was kept constant at a 12 : 12 h light/dark cycle. Songs were saved automatically 24 h per day using the SOUND ANALYSIS PRO program (v1.04; http://ofer.sci.ccny.cuny.edu) (Tchernichovski *et al.*, 2000) at 16 bits and a 44-kHz sampling rate. To obtain high-quality recordings for quantitative analysis, we used sensitive microphones (SRO, Earthworks) with a flat frequency response up to 20 kHz, as recommended in the SOUND ANALYSIS PRO user manual. We used animals that spontaneously sang at least 100 song bouts per day. After the 3–7 days of recording, a subgroup of birds was deafened ($n = 9$) and then placed back into the sound-attenuation chambers and songs were recorded for 14 days thereafter. Intact controls ($n = 6$) were kept in the sound-attenuation chambers for at least another 14 days. We also conducted experiments on sham controls ($n = 3$) that underwent surgery but not deafening. In their last recording session, after 30 min of singing, all birds were killed and singing-driven immediate-early gene (IEG) expression in song nuclei (Jarvis & Nottebohm, 1997; Wada *et al.*, 2004) was analysed for a related study (unpublished).

Song acoustic analysis

Analysis of syllable acoustic structure was performed using the SOUND ANALYSIS PRO program. We measured syllable Wiener entropy, Wiener entropy variance, pitch, frequency modulation (FM) and amplitude (Tchernichovski *et al.*, 2000). For all analyses, we used $n = 30$ examples of each song syllable from each bird per time-point. To select these syllables, we scanned through digitized song spectrograms generated by SOUND ANALYSIS PRO in the first 1–2 h after the lights were turned on and selected the first syllables produced in song bouts that did not have overlaid sounds of mechanical noise due to the bird hopping or distortion due to the bird being too close (usually within 1–3 cm) to the microphone. We filtered out low-frequency noise (< 1 kHz), reduced mechanical noise from the recordings of some birds by using AVISOFT-SASLAB (Avisoft Bioacoustics, Germany) and further filtered low-frequency noise (< 1.5 kHz) by SOUND ANALYSIS PRO. We excluded introductory notes from the syllable acoustic analyses, as they were quite variable in each song bout even before deafening. A sample size of 30 per time-point was sufficient for statistical analysis to show significant differences. From the 14 days of recording, we used three time-points for the analysis, i.e. days 3, 8 and 14. Day 3 was the first day that all of the birds sang a sufficient amount of song for quantitative analysis and day 14 was the day of killing. Samples were taken from recordings at 1–2 h after lights were turned on, in order to be cautious about possible unknown circadian effects in song behavior (Deregnaucourt *et al.*, 2005; Glaze & Troyer, 2006). Because entropy variance differs for each syllable of a bird, for time-course analysis we normalized the values of syllables by dividing the entropy variance of each syllable by the value 2–3 days before deafening. We then averaged the ratios for all syllables of each bird to obtain an average value of change for each bird. A ratio

of ~1 indicates no change in the variance, a ratio > 1 indicates an increase in variance and < 1 indicates a decrease.

For some syllables we further analysed their mean FM and harmonic structure. To determine differences across birds, we first calculated the mean FM from 30 examples of each syllable for each bird, reordered the syllable values from the lowest to highest FM, averaged the values in this order across birds and then determined if there were statistical differences of a specific syllable type relative to all other syllables, using ANOVA with bird as a factor. Because the number of syllable types in a bout was different for each bird (total of three to six), the average FM values across birds contained syllables from some or all birds. To determine whether a syllable had harmonic structure, we used AVISOFT-SASLAB to calculate the peak frequencies in the power spectrum of the syllable (n = 5 syllables of one type from five deafened birds). The first peak was designated as the fundamental frequency (F_0). The values of the subsequent peaks (up to five peaks: F_1–F_5) were then divided by the F_0 and assessed as to whether these were whole integer ratios of the F_0. If so, then the syllable was considered as harmonic.

Song sequence analyses

Song sequence analyses were conducted on 30–40 song bouts per time-point per bird using seven measures.

(1) Mean number of syllables produced per bout for syllables that were subsequently deleted due to deafening. For these syllables, we counted the number of syllables of that type per bout per day.
(2) Mean inter-motif interval length per bout (a temporal but not necessarily a sequence measure). We measured the duration (in ms) from the end of the first motif to the beginning of the next syllable, whether or not it was part of the next motif (i.e. inter-motif syllables or long calls after the motif), and calculated the average interval per bout per day.
(3) Mean number of motifs per bout. We counted the number of motifs in each bout and then calculated the average number of motifs per bout per day.
(4) Mean syllable repetition per bout. For the same syllable that was repeated consecutively within a song bout, we counted the number of repetitions per bout per day.
(5) Sequence transition probability diagrams of song bout sequences were created following Ferreira *et al.* (2006). Briefly, we determined the total number of each song syllable transition type (e.g. A to B, B to C, B to A, A to A, etc.) across the 30–40 song bouts and divided that number by the total number of all transitions to obtain a probability (range 0–1) for each transition type. These probabilities were then graphed in a sequence transition diagram, where thicker and warmer colored arrows indicate more frequently produced syllable transitional probabilities, arrows that curve backwards from the end to the beginning of a sequence represent repeated motifs, and vertical arrows pointing downwards to horizontal lines designate song bout stop points.
(6) Sequence linearity was calculated following the equation of Scharff & Nottebohm (1991)

$$\text{sequence linearity} = \frac{(\text{number of syllable types/bout})}{(\text{number of transition types/bout})}.$$

(7) Sequence consistency was also calculated following Scharff & Nottebohm (1991)

$$\text{sequence consistency} = \frac{(\text{number of typical transitions/bout})}{(\text{total number of transitions/bout})}.$$

For both sequence linearity and consistency measures, we included all syllables of the song bout, including introductory, inter-motif and post-motif syllables. The closer to 1 for both values, the more stereotyped the linearity or consistency of the song bout sequence. In previous studies (Foster & Bottjer, 2001; Kao & Brainard, 2006), introductory syllables were sometimes not included in these measures, which results in higher stereotypy values for the remaining song, as introductory syllables are produced in more variable amounts from bout to bout. We decided to include introductory syllables in sequence analyses because we found that deafening had an effect on the frequency of their production.

To calculate the relative rates (R) of changes for all measures (acoustic, sequence and temporal) within different periods over the 2 weeks, we used the following equation

$$R = \log \frac{V_{14d} - V_{3d}}{V_{3d} - V_{0d}}$$

where V_{xd} is the value of the variable measured on a given day and x is the day. These rates for all seven measures and the measures themselves are not all independent of each other. In particular, sequence linearity, sequence consistency and the values and structure of the transition diagrams depend on sequence measures (1), (3) and (4). These three measures, however, may or may not be independent, as they depend on neural mechanisms of song production.

Surgical manipulations

Deafening

Animals were deafened by cochlear removal using a previously described procedure (Konishi, 1964; Lombardino & Nottebohm, 2000), which we learned from Dr Wan-chun Liu (The Rockefeller University, NY, USA). Birds were anesthetized with Nembutal (10 mg/mL; 40 μL/12 mg of body weight). After stabilizing the head in a custom-built rotating operation stage or a stereotaxic apparatus with ear bars, a small window was made through the neck muscle and the skull, underlying the end of the elastic extension of the tongue bone. A small hole was then made in the cochlear dome. Through this hole, the cochlea was pulled out with a fine hooked wire. After bilateral cochlea removal, the skin incisions were sealed with cyanoacrylate and the bird allowed to recover for 24 h. All birds started to sing again within 72 h.

Brain lesions

Young adult males were treated in a similar manner as for the deafening experiments except that, after the 3–7 days of pre-surgical recordings, LMAN (n = 10) or MMAN (n = 7) was lesioned bilaterally. The birds were then placed back into the sound-attenuation chambers and allowed to recover while song was recorded for 3–5 days. Of these birds, two LMAN-lesioned and one MMAN-lesioned bird stopped singing during the 3–5 days of recordings after receiving lesions. As they did not sing during this 3–5 day period, we stopped further manipulations of them and did not examine their lesions. For the remaining birds (n = 8 LMAN lesioned; n = 6 MMAN lesioned), after they sang a sufficient number of song bouts (at least 50) for 1–2 h in the morning within 3–5 days post-lesion, we deafened them that night, placed them back into the sound-attenuation chambers and recorded their songs for 14 days. All of these birds sang after deafening. In their last recording session, after 30 min of singing, all birds were killed and singing-driven IEG expression in song nuclei was analysed. We used the singing-driven IEG expression to determine if any remaining neural tissue was functional and thus the lesion size (described below).

LMAN and MMAN lesions

Birds were anesthetized with ketamine/xylazine (15 mg/mL ketamine, 3 mg/mL xylazine; 40 μL/12 mg of body weight). LMAN and MMAN were targeted based on stereotaxic coordinates [LMAN range (mm): 4.0–4.6 rostrally, 1.5–1.8 laterally, 1.9–2.5 ventrally; MMAN range: 4.0–4.6 rostrally, 0.3 laterally, 1.9–2.2 ventrally from the bifurcation of the central sinus at the border of the forebrain and cerebellum] as well as on electrophysiological activity for some animals. Spontaneous activity of these song nuclei is different from the surrounding brain areas, allowing us to locate them. To record this activity, we used a tungsten electrode. Once the vocal nucleus was found, we replaced the electrode with another that had a scraped tip. Electrolytic lesions were then performed with three to five penetrations for LMAN and two to three for MMAN per hemisphere (combination of dorsal-ventral and medial-lateral locations in each nucleus) and three (LMAN) or one to two (MMAN) current injections per penetration (200 μA, 30–40 s).

The LMAN or MMAN lesion size and remaining function were assessed by two measures, i.e. Nissl staining and singing-driven IEG expression. A previous study has shown that, when part of LMAN or MMAN is lesioned, the remaining part is still functionally active during singing (Kubikova *et al.*, 2007). To perform these measures, after their last recording session, birds were housed alone overnight for > 12 h of silence. After lights were turned on, they were allowed to sing for at least 30 min. Brains were dissected, placed in a tissue block mold, covered in TissueTek (Sakura Finetek), frozen in crushed dry ice and sectioned with a cryostat in the sagittal plane at 12 μm thickness. ^{35}S-riboprobe *in-situ* hybridizations with egr-1 (also known as ZENK) and mkp-1 were conducted following a previously described procedure (Wada *et al.*, 2006). Both IEGs are induced in LMAN and MMAN following singing (Jarvis & Nottebohm, 1997; our unpublished results), where the activation of mkp-1 is highly specific to LMAN. Slides were exposed to X-ray film (β-max hyperfilm, Kodak) for 2 (egr-1) or 2–5 (mkp-1) days. The slides were dipped in photo-emulsion (NBT2, Kodak), exposed for 1–2 weeks, developed and sections stained with cresyl violet. Lesion size was determined by dividing the difference of the remaining proportion of functional LMAN or MMAN that showed egr-1 and mkp-1 gene expression by the average size of these nuclei in intact animals (as determined by Nissl stain and IEG expression), multiplying by 100 and averaging between the two hemispheres to obtain percentage lesion size for each animal.

Statistics

To determine if differences in syllable structure (e.g. entropy) were significant before and at 2 weeks after deafening, we used ANOVA followed by a Fisher's *post-hoc* test, with bird and syllable as factors. This essentially resulted in paired comparisons within bird and within syllable of each bird. For time-course analysis of syllable structure and syllable sequence changes, we determined if differences were significant within each bird using a paired *t*-test for each time-point. To determine if there were significant differences in the rates (R) of change, we performed ANOVAs followed by Fisher's *post-hoc* test.

Results

To test our hypothesis that medial and lateral divisions of the anterior vocal pathway function differentially for deafening-induced modification of song and to quantitatively characterize deafening-induced changes in the early stages of deafening, we performed two types of experiments: (i) deafening experiments in young adult animals to determine quantitative time-course analyses of changes in vocal behavior and (ii) LMAN and MMAN lesion experiments to determine the functional requirements of these nuclei for the deafening-induced changes.

Deafening experiments

Deafening-induced changes to song syllable structure

After 2 weeks, deafened young adult birds showed significant increases in Wiener entropy of most syllables (Fig. 2A and B, top panels). Associated with increased entropy were significant decreases of entropy variance within each syllable (Fig. 2A and C), i.e. the syllables became noisier (entropy) and the noisiness was relatively uniform within each syllable (entropy variance). Qualitatively, these changes in syllable spectral structure did not affect syllable recognition in human observers (Fig. 2A) but, quantitatively, the changes were significantly robust (Fig. 2B and C). The entropy changes occurred gradually and began as little as 3 days after deafening, when the birds began to sing a sufficient number of songs (Fig. 2D). Intact animals did not show significant changes in entropy or entropy variance within the same time period (Fig. 2A–D, lower panels). We did not detect any significant changes in syllable pitch ($P = 0.372$), FM ($P = 0.342$) or amplitude ($P = 0.681$) in the deafened young adult animals (paired *t*-test with each song syllable before and after 2 weeks). These findings suggest that song syllable structure changes in young adults begin to occur very soon after deafening, entropy variance is a sensitive measure of these changes that could be missed by other quantitative or qualitative analyses, the entropy change is gradual, and these changes are not a reflection of other measured acoustic features, such as amplitude.

Deafening-induced vocal changes to song bout sequence and temporal structure

The deafened young adults did not show significant changes in song motif sequences within 2 weeks as previously reported (Lombardino & Nottebohm, 2000) but a number of changes occurred in broader aspects of syllable sequencing, i.e. song bout sequencing and temporal structure. We detected at least seven types of changes in song bout sequencing or temporal structure (Figs 3–6): (i) dropping of inter-motif syllables; (ii) increases in inter-motif interval lengths (temporal change); (iii) decreases in the number of motifs produced per bout; (iv) stuttering-like repetition of some syllables; (v) changes in probabilities of song bout sequence transitions; (vi) changes in song bout sequence linearity; and (vii) changes in song bout sequence consistency.

For dropping of syllables, we noted that there exists a certain type of inter-motif syllable that we call a glue syllable, which was dropped in those birds that produced such a syllable before deafening (Fig. 3A; three of six intact and five of nine deafened birds had such a syllable). This syllable in every bird ($n = 8$) had a characteristic relatively flat frequency stack structure with statistically significantly lower FM relative to all of the other syllables in the song motif (Fig. 4A–D). The stack frequencies in these syllables were harmonics of the fundamental frequency (Fig. 4E), i.e. all such glue syllables were harmonic stack syllables (Fig. 4E) but not all harmonic stack syllables were glue syllables. The glue syllables appeared between motifs and sometimes also appeared at the end of a motif as the last syllable of a song bout (Fig. 3B). In some birds ($n = 2$ of 5 deafened animals with glue syllables), the glue syllable was dropped together with one or more adjacent syllables also produced along with the glue syllable as inter-motif syllables

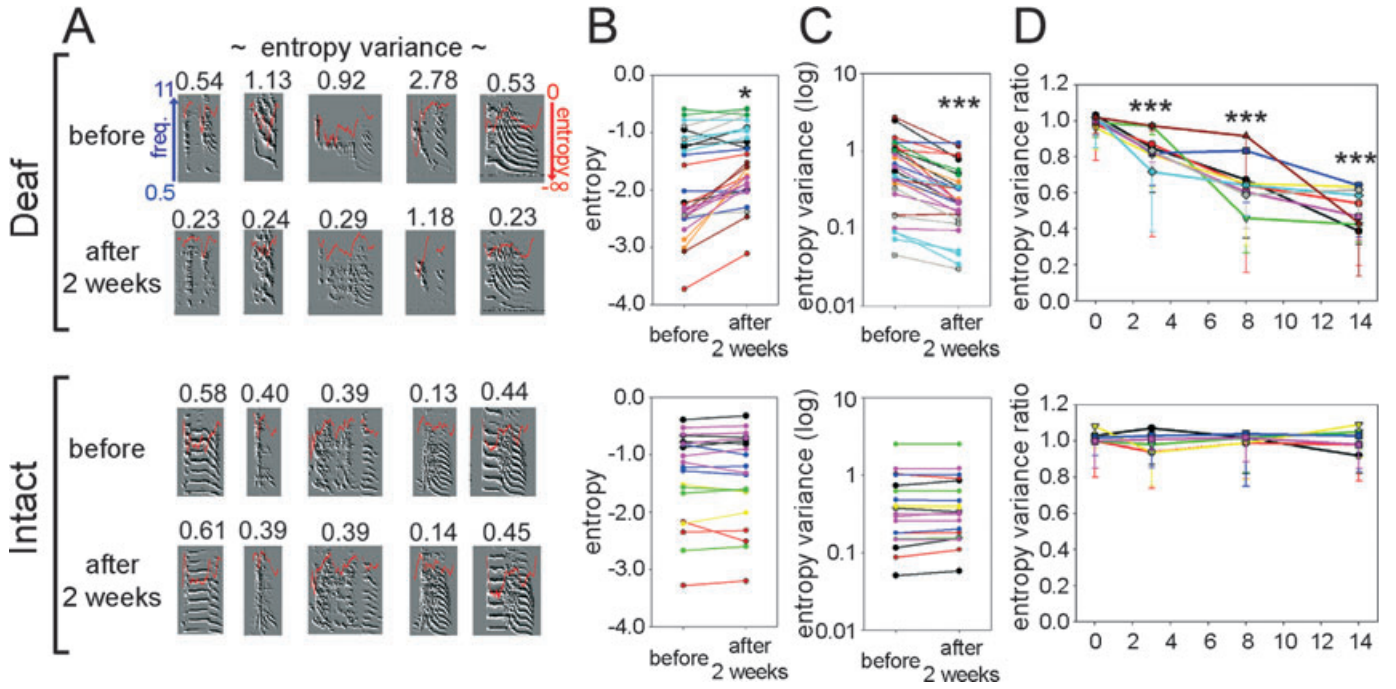

FIG. 2. Rapid deterioration in syllable spectral structure after deafening. (A) Examples of syllables in deafened (top panels) and intact (lower panels) birds before (for deafened animals) and at 2 weeks after deafening. Red line on each syllable spectrum is the entropy across time for that one syllable rendition (the closer to 0, the more entropy). The number above each syllable is the value of the mean entropy variance within that one syllable. (B) Mean entropy in each syllable of birds before and at 2 weeks after deafening. Each color indicates one bird and multiple values of the same color indicate different syllables of the same bird (average syllables that exist after deafening per bird = 3.26). (C) Mean entropy variance in each syllable of all birds before and at 2 weeks after deafening; color coding is the same as in B. $*P < 0.05$, $***P < 0.001$, ANOVA, Fisher's *post-hoc* test, with bird and syllable as factors. (D) Time-course of changes in mean entropy variance of syllables before (0) and on subsequent (3, 8 and 14) days after deafening, averaged across all syllables within each bird as ratios (bars are SD). Entropy variance ratios were created by normalizing values for each syllable with values from 2–3 days before deafening. $***P < 0.001$; paired *t*-test with 0 time-point; $n = 9$ deafened birds, $n = 6$ intact birds, $n = 30$ syllables each.

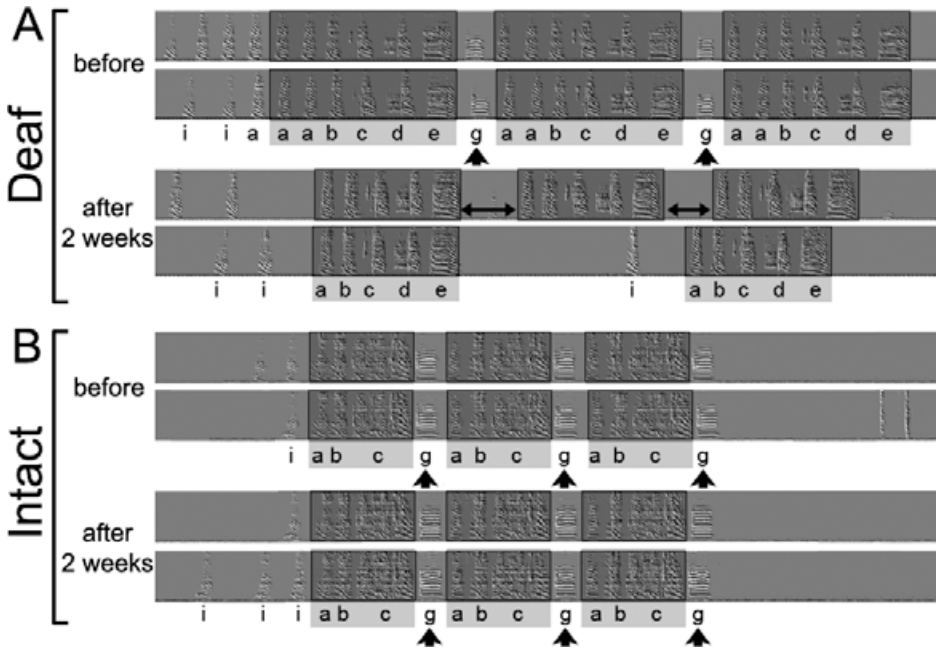

FIG. 3. Examples of the most common deafening-induced song bout sequence and temporal changes. (A) Song spectrograms before and at 2 weeks after deafening, showing dropped glue (g) syllables (thick arrowheads), increased inter-motif intervals (double-ended arrows) and fewer motifs (boxed sequences) per bout. (B) Song spectrograms of an intact animal within the same time period. i, introductory syllables.

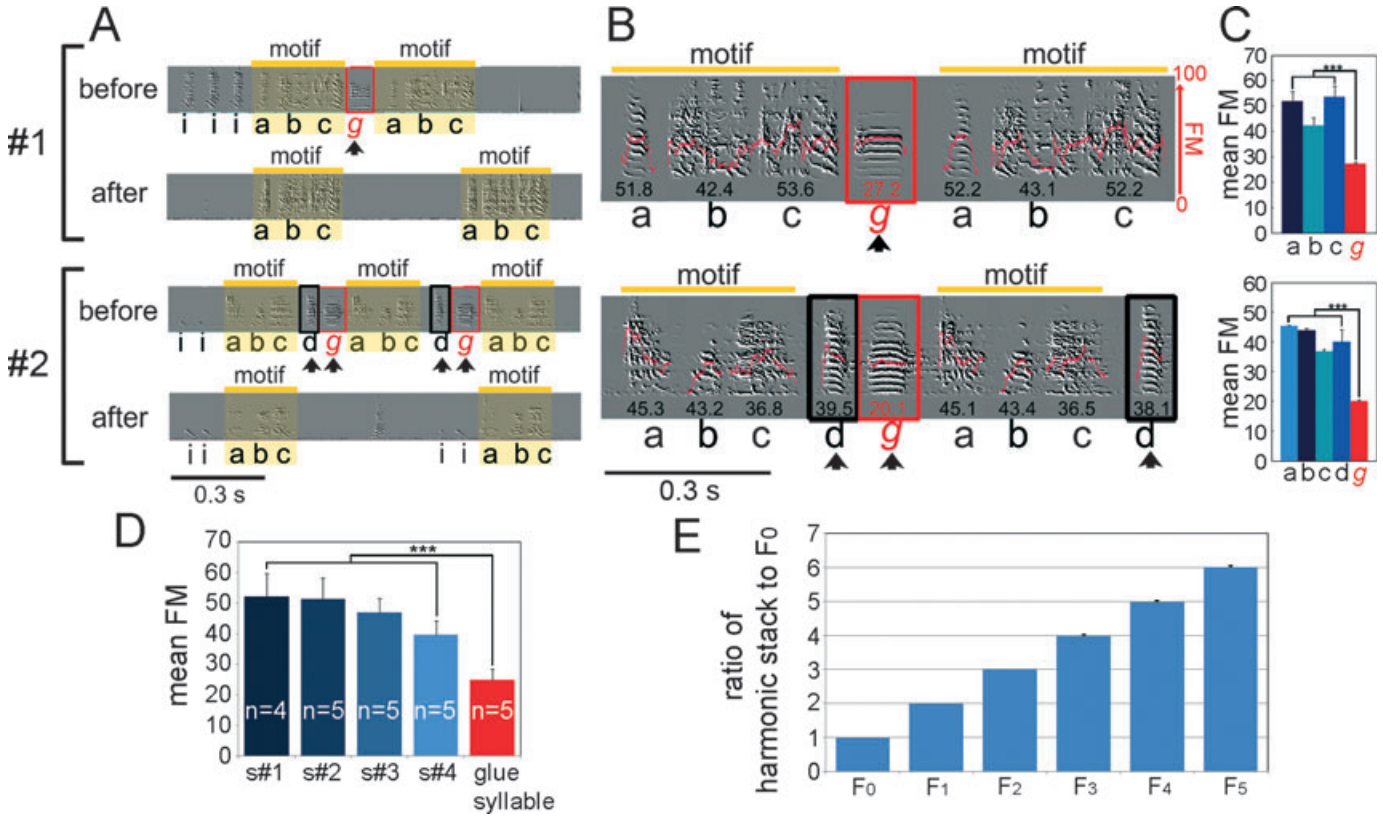

FIG. 4. Characterization of glue syllables. (A) Song spectrograms of two example birds with glue syllables before and after deafening. In example no. 1, only the glue syllable (*g*) (red bracket) was dropped after deafening. In example no. 2, the glue (*g*) (red bracket) and adjacent (d) (black bracket) syllables were dropped. Yellow color highlights the stable motif. i, introductory syllables. (B) Magnified song spectrograms of song bouts from birds 1 and 2 with glue syllables before deafening. Red line is the mean FM across time for each syllable and the number below is the mean FM for the entire syllable. (C) Mean FM of each syllable across multiple song bouts ($n = 30$) for the two example birds (nos 1 and 2). Glue syllables in their songs have significantly lower FM relative to other syllables. (D) Mean FM of up to four syllables (except for one bird with three) from the five deafened birds that produced glue syllables, ordered from highest to lowest FM, regardless of order produced in song (n = number of birds with such a number of syllables). Similar to the example birds, the glue syllable in all birds had the lowest FM. (E) The stack structure of the glue syllables are harmonics (F_1–F_5) of the fundamental frequency (F_0; $n = 5$ glue syllables from five deafened birds each). Shown is the average ratio across birds of the higher frequency bands (F_1–F_5) to the F_0. For all graphs, ***$P < 0.001$; ANOVA, Fisher's *post-hoc* test.

(Fig. 4A, example no. 2). Unlike glue syllables, these adjacent syllables had similar FM to most motif syllables (Fig. 4B and C, example no. 2). In one bird, several of these inter-motif syllables were also present in the motif (syllables b and c in Fig. 5A). Dropping of glue and adjacent syllables occurred simultaneously and rapidly (within 3 days) after deafening, followed thereafter by further gradual decreases in the frequency of production of those syllables (Fig. 6A, a, $n = 5$). Intact controls did not show a change in the frequency of production of glue syllables over the same time period (Fig. 6A, b, $n = 3$).

Associated with the dropping of inter-motif syllables, there was an increase in the silent intervals between motifs (Figs 3A and 6A, c, when including the glue syllable as part of the motif). Birds that did not produce glue syllables before deafening, except for one, did not show increases in inter-motif intervals (Fig. 6A, a and c, # in A, c is the exception), i.e. there was no condensing of time between motifs when the syllables were dropped. In addition to these changes, all birds with or without glue syllables showed a decrease in the number of motifs produced per song bout such that, by the end of 2 weeks, several of them produced almost only one song motif per bout most of the time (Fig. 6A, e). Interestingly, intact controls did not show a decrease in the number of motifs per bout as expected but each bird produced a stable average number of motifs per bout that was relatively unique for that bird (Fig. 6A, f). This stability was best revealed when averaging across many ($n = 30$) song bouts per bird.

Another type of sequence change was in syllable repetition. Some birds ($n = 4$ of 9) sequentially repeated a syllable(s) outside the motif sequence before deafening (Fig. 5B). After deafening, these birds showed either a gradual increase or decrease in repetition of those syllables (Fig. 6A, g). The increased repetition sounded like stuttering. Birds that did not have repetitive syllables before deafening, or had repetitive syllables and were not deafened ($n = 3$ of six intact animals), did not show changes in syllable repetition (Fig. 6A, h). However, another overlapping subset of birds ($n = 4$ of 9), whether or not they produced repetitive syllables before deafening, had increased the repeated production of calls at the end of a bout (Fig. 5B, f' syllables).

Global effects of changes in song bout sequencing were captured in sequence transition probability diagrams (Fig. 5A–C, lower panels), and sequence linearity and sequence consistency measures (Fig. 6A, i–l). In birds with glue and other inter-motif syllables, the most prominent sequence change in the transition probabilities by 2 weeks was the dramatic reduction of these syllables and the reduction of transitions to another complete motif after the first complete motif within a bout (Fig. 5A and B, lower panels). In birds with repetitive syllables before deafening, frequency of repetition was a common change but the motif sequence was maintained (Fig. 5B). In one bird

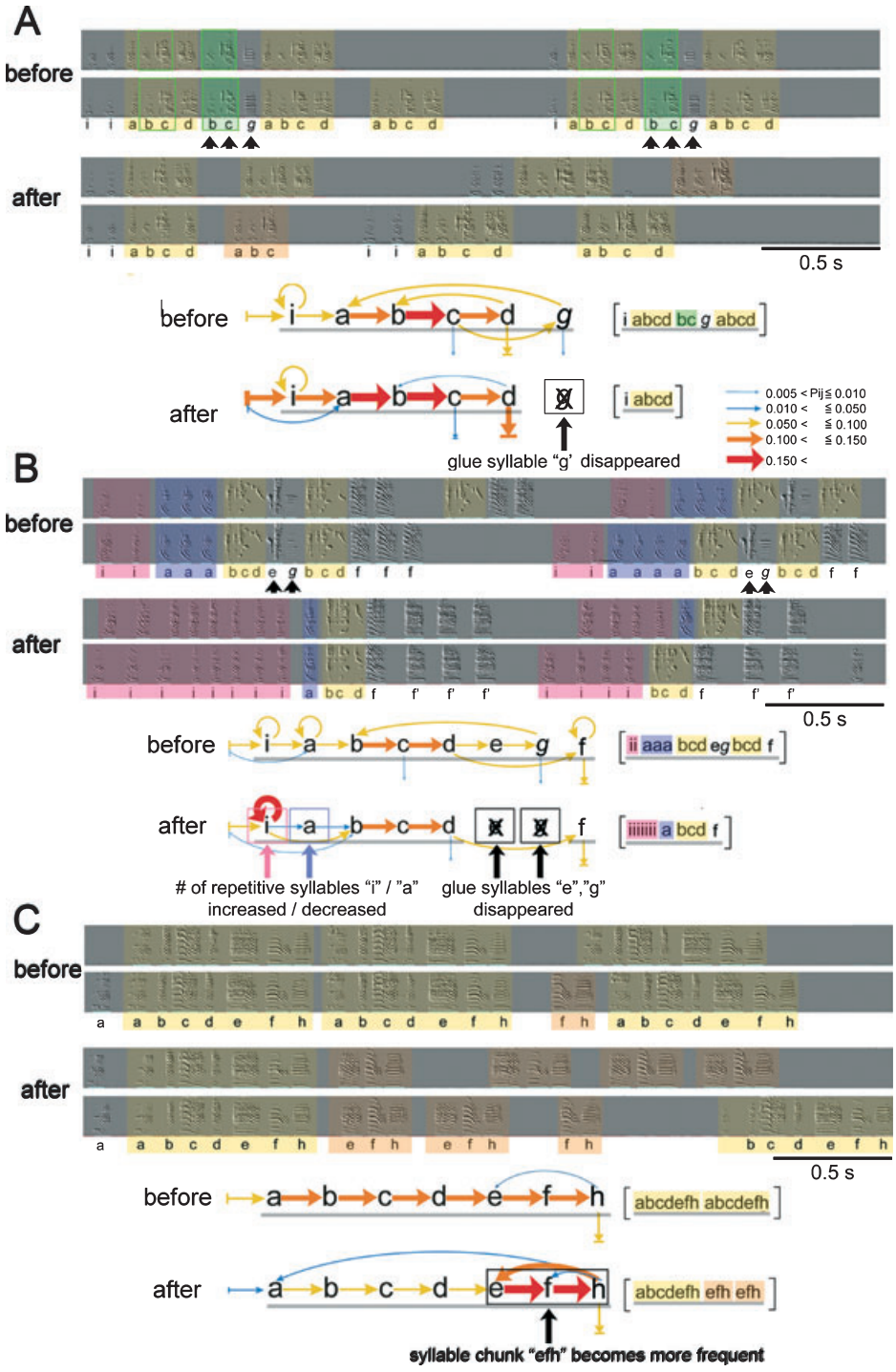

FIG. 5. Example spectrograms and sequence transition diagrams of the deafening-induced song bout sequence changes found. In each example shown, song spectrograms are in the upper panels and transition diagrams are in the lower panels, for before and after deafening. In addition, the most frequent sequence is shown to the bottom right in square brackets. In the transition diagrams, the probability (P_{ij}) of the transition from syllable *i* to syllable *j* is represented with line thickness and colored arrows (higher P_{ij}, thicker line and warmer color; $n = 30$ song bouts per bird, before and after deafening). Yellow colored sequence is the motif, orange is other syllable chunks, pink is increased repetitive syllables and blue is decreased repetitive syllables. (A) Example bird with glue syllables and adjacent syllables (b and c) that were dropped. (B) Example bird with repetitive syllables with increased (i, introductory) or decreased (a) syllable repetition. (C) Example bird with a chunk of motif sequence (efh) that was repeated more often after deafening.

($n = 1$ of 9), chunks of syllables that were rarely separated from the motif before deafening were produced at a much higher probability after deafening (Fig. 5C).

All birds, except for one, showed an increase in sequence linearity (number of syllable types per bout/number of syllable transition types per bout) after deafening (Fig. 6, i, # is the exception; also in Fig. 5C). In contrast, for sequence consistency (number of dominant syllable transitions per bout/total number of syllable transitions per bout) about half of the birds showed an increase and the other half showed a decrease (Fig. 6A, k). The major factor for the increased sequence

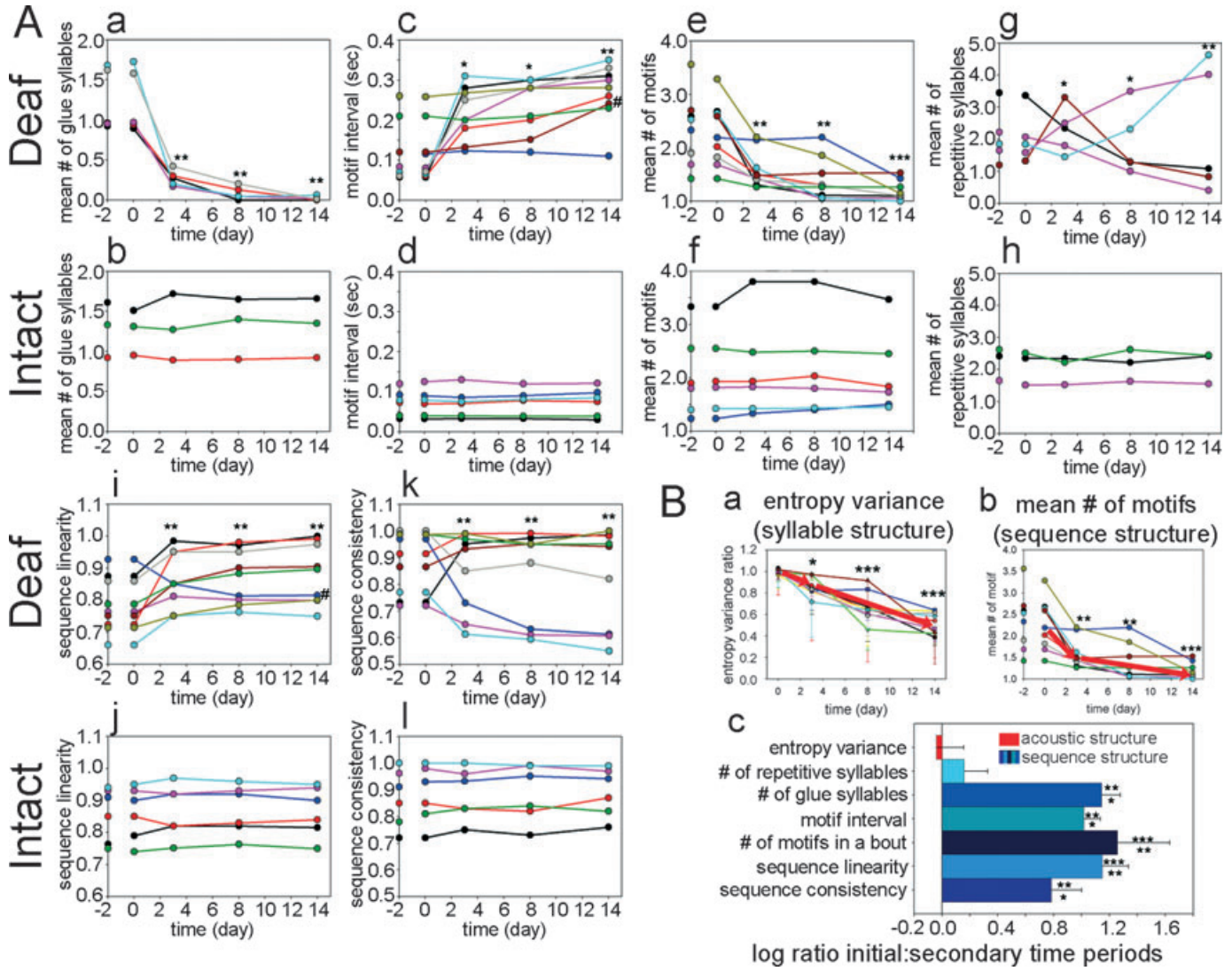

FIG. 6. Quantification and time-course analysis of deafening-induced changes in song bout sequencing. (A) Graphs for six of the seven song bout sequence changes measured in deafened (a, c, e, g, i and k) and intact (b, d, f, h, j and l) birds. Colors indicate the same bird for deaf or intact animals. For each graph, the value at −2 days (on *y*-axis) before deafening is shown for comparison; 0 is the morning before deafening. Values represent averages from the 30 song bouts at each time-point. $*P < 0.05$, $**P < 0.01$ $***P < 0.001$; paired *t*-test relative to −2 days. SD bars are not shown, as they make the figures cumbersome to follow, and nor are statistical analyses shown for each individual bird. For (c), birds with glue syllables were included as one group for statistical analysis. For sequence features where deafening-induced changes went in opposite directions for different birds (g, i and j), we used absolute values to determine statistical differences for the group of birds. (B) Examples of average slopes for entropy variance (a) and mean number of motif changes in a bout (b) over time. Thick red lines indicate average slopes of two different time periods (initial and secondary) for illustrative purposes. Graphs are from Fig. 2C and panel A (e) of this figure, respectively. (c) Log of the rates (ratios) of changes (differences) from initial and secondary time periods for seven of the deafening-induced changes measured. A log ratio of 0 indicates a smooth change across time; < 0 indicates a slow change followed by a more rapid change; > 0 indicates a rapid change followed by a slower change. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, relative to entropy variance (above SEM bars) and number of repetitive syllables (below SEM bars); ANOVA, Fisher's *post-hoc* test.

linearity was fewer motifs (and thus transitions) per bout; a second additive factor was for birds with glue syllables due to loss of more transitions relative to the number of syllables per bout. The factor contributing to increased syllable consistency was fewer dominant syllable transitions due to dropping of glue syllables and the factor contributing to decreased syllable consistency was increased modulation of repetitive syllables due to an increased number of syllable transitions in birds that had repetitions; a decreased production of repetitive syllables had the opposite effect. The one bird with decreased sequence linearity was due to an increased probability of producing syllable chunks, and thus an increase in the number of transition types, resulting in a less stereotyped song. The time-course of the sequence linearity and sequence consistency changes occurred rapidly after deafening and was then maintained for the 2 week period (Fig. 6A, i–l).

To determine if there were significant differences in the time-course of the different deafening-induced changes, we measured the rates (i.e. ratios) of the differences in the changes within the first few days relative to the remaining days after deafening (Fig. 6B, a and b). We found that changes in entropy and syllable repetition occurred gradually, whereas all other changes occurred more rapidly (Fig. 6B, c). These differences in the rates of changes were significant (Fig. 6B, c). These findings indicate that changes in syllable repetition (which is gradual) contribute less to the sequence linearity changes than do changes in the numbers of glue syllables and motifs (which are rapid). These findings suggest that different neural mechanisms

contribute to changes in syllable structure and sequence, an idea that we tested next.

Deafening and brain lesion experiments

We tested whether LMAN and MMAN were differentially required for deafening-induced changes in syllable structure and sequencing as hypothesized (see Introduction), by first lesioning each nucleus and then deafening the animals. To determine that LMAN and MMAN were functionally lesioned, we assayed for singing-driven gene expression of egr-1 and mkp-1. We found that LMAN lesions did not prevent gross singing-driven gene expression in MMAN or other connected song nuclei (Fig. 7A). The same was true for MMAN lesions (Fig. 7B and C). These findings are consistent with previous experiments that showed that LMAN or MMAN lesions lead to the same or higher singing-driven gene expression in the connected song nuclei (Kubikova *et al.*, 2007).

LMAN but not MMAN is required for the deafening-induced changes to syllable structure

Figure 8 shows values of entropy variance in lesion-deafened animals (compare with Fig. 2). Lesions to LMAN or MMAN alone did not result in any rapid (3–5 days) changes in syllable entropy variance (Fig. 8C). When these animals were subsequently deafened (in six of eight animals), LMAN lesions reduced or blocked the deafening-induced syllable structure changes in entropy variance (Fig. 8A–C, top panels). The two birds that did show a significant decrease (# in Fig. 8C) had the smallest lesions (one had a bilateral average of 50% lesioned and the other was 70% lesioned). Consistent with these findings, there was a significant negative correlation between LMAN lesion size and change in entropy variance (Fig. 9A); deaf birds with lesions outside LMAN ($n = 2$) showed changes in entropy variance as large as those in LMAN-intact animals (Fig. 9A, open circles with dots on y-axis). In contrast, MMAN lesions did not prevent the deafening-induced syllable structure changes in entropy variance (Fig. 8A–C, bottom panels). The changes in entropy variance in the deaf MMAN-lesioned animals were similar to those seen in deaf MMAN-intact animals [$P = 0.598$ (0 days), 0.665 (3 days), 0.341 (8 days), 0.690 (14 days), *t*-test]. Further, there was no correlation between MMAN lesion size and changes in entropy variance (Fig. 9B).

LMAN and MMAN are not required for the deafening-induced changes to song bout sequence

In the same animals as described above, lesions to LMAN or MMAN alone did not result in any noticeable rapid (3–5 days) changes in the seven sequence features that we measured (Figs 10 and 11). In fact, the overall probabilities of song bout sequencing were rather stable before and after the lesions (Fig. 10, before and after LMAN lesions). When these animals were subsequently deafened, deafening-induced changes occurred in the seven sequence/temporal measures. These included: dropping of glue and associated syllables in birds that produced them before the lesions (Figs 10A–C, and 11A and B); associated increases in inter-motif intervals (Figs 10B, and 11C and D); decreases in the number of motifs produced (Figs 10A–D, and 11E and F); increases or decreases in the production of repetitive syllables (Figs 10B, and 11G and H); changes in probabilities of song bout sequence transitions (Fig. 10A–D); mostly increases in sequence linearity (Fig. 11I and J); and changes in sequence consistency (Fig. 11K and L). In summary, lesions to LMAN in young adult birds prevented the deafening-induced changes in syllable acoustic structure (i.e. entropy) but did not prevent the changes in song bout sequencing. Further, lesions to

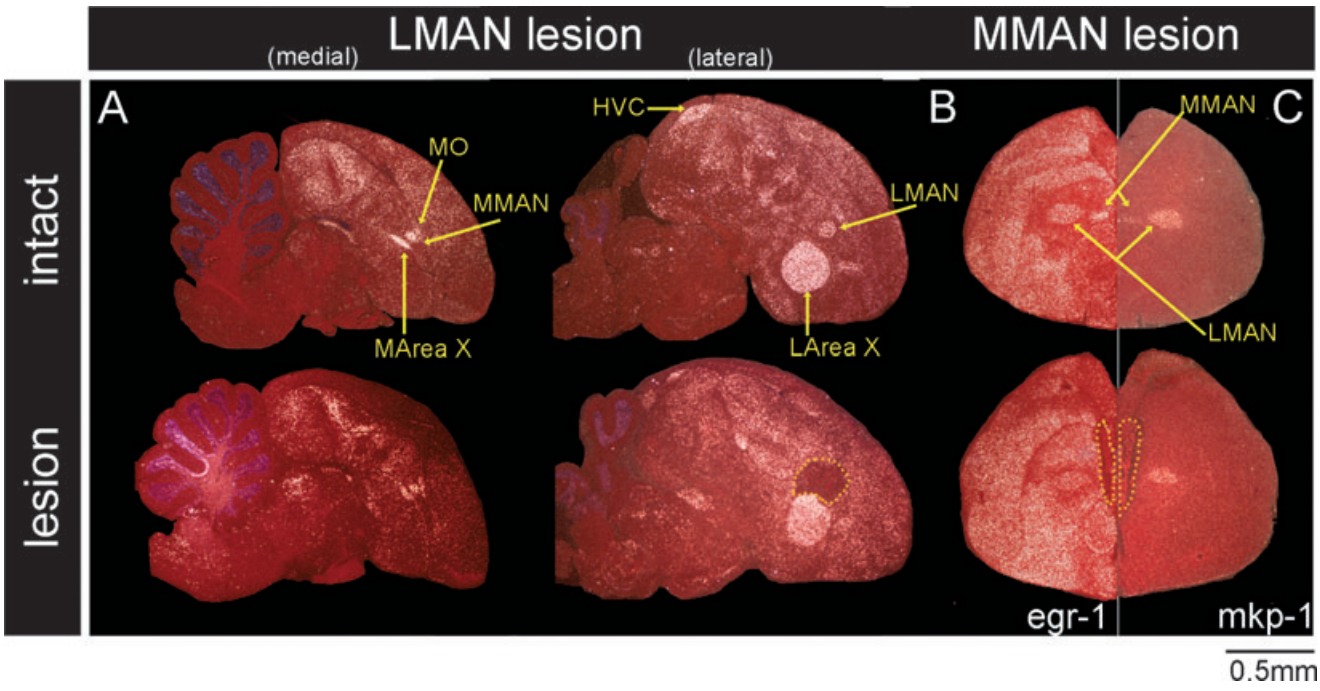

FIG. 7. Extent of LMAN and MMAN lesions revealed by singing-driven egr-1 and mkp-1 expression. (A) Example sagittal sections of deafened animals with LMAN either intact (top) or lesioned (bottom). Singing-induced egr-1 expression can be seen in MMAN, LMAN, Area X and HVC of the LMAN-intact deaf animal but not in LMAN of the LMAN-lesioned animal. Anterior is to the right and dorsal is up. (B and C) Frontal sections of deafened animals with MMAN intact (top) or lesioned (bottom). Singing-induced egr-1 (B) and mkp-1 (C) expression can be seen in MMAN and LMAN of the intact animal but not in MMAN of the MMAN-lesioned animal. B and C are adjacent sections hybridized to the two genes separately. Medial is in the center and dorsal is up. White, gene expression; red, Nissl stain; dashed yellow lines, boundaries of lesion sites. Scale bar, 0.5 mm. LAreaX, lateral AreaX; MAreaX, medial AreaX; MO, oval song nucleus of the mesopallium.

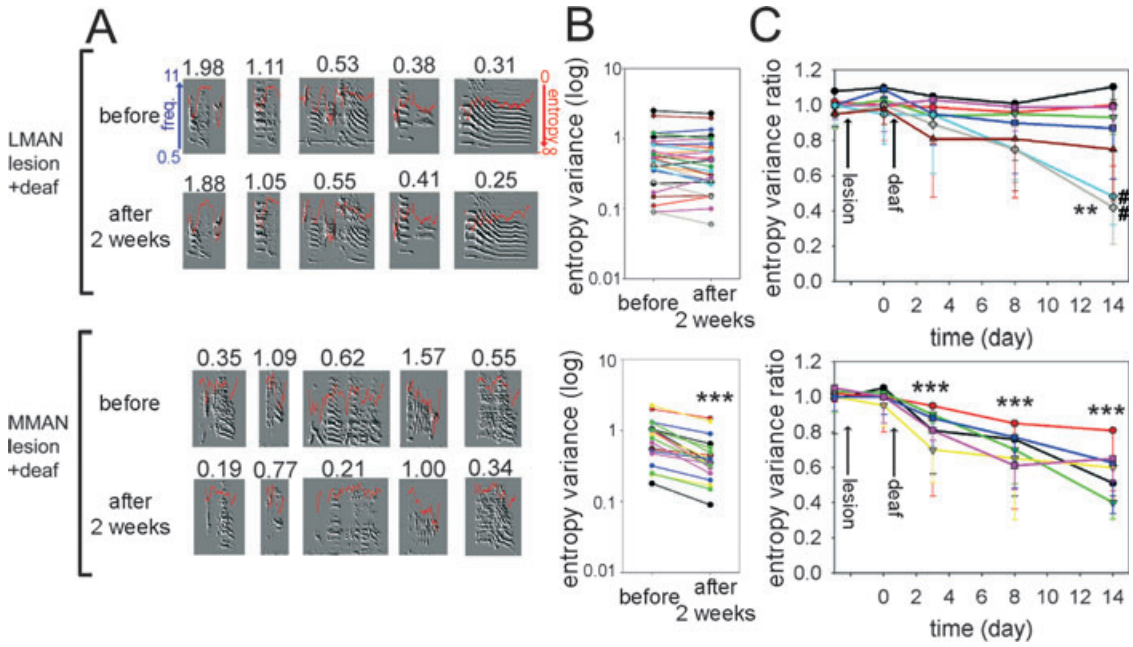

FIG. 8. Syllable spectral structure after anterior song nuclei lesions and deafening. (A) Examples of changes in syllable entropy variance in LMAN- (top) and MMAN- (bottom) lesioned birds before and at 2 weeks after deafening. Red line on each syllable spectrum is the entropy across time for that one syllable rendition. The number above each syllable is the value of the mean entropy variance within that one syllable. (B) Mean entropy variance in each syllable of all lesioned birds at 3–5 days before and 14 days after deafening. Each color indicates one bird and multiple values of the same color indicate different syllables of the same bird. ***$P < 0.001$, ANOVA, Fisher's PSLD *post-hoc* test, with bird and syllable as factors. (C) Time-course of changes in mean entropy variance of syllables at 3–5 days before lesion (first value), 3–5 days after lesion (0) and after deafening (all other values). Entropy variance ratios were created by normalizing values for each syllable with the respective values from 3 to 5 days before lesioning. Arrows indicate relative time that lesioning and deafening were performed. **$P < 0.01$, ***$P < 0.001$, paired *t*-test; # indicates two birds that showed a significant decrease in entropy variance (paired *t*-test within bird using syllables as variables); $n = 8$ LMAN lesion + deafened birds, $n = 6$ MMAN lesion + deafened birds, $n = 30$ syllables each. Bars are SD.

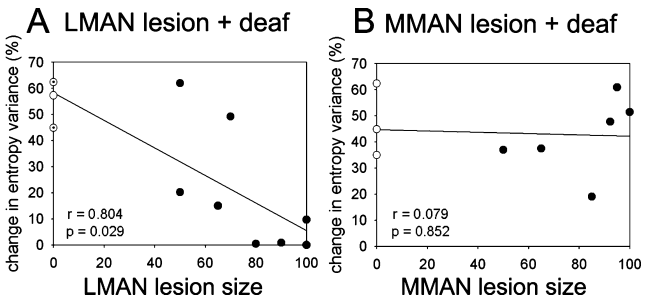

FIG. 9. Relationship between lesion size and change in entropy variance. (A) LMAN-lesioned + deafened birds ($n = 8$) and LMAN-intact birds ($n = 3$). Two of the LMAN-intact birds (open circles with a central dot on the *y*-axis) had either an electrode placed in LMAN but no lesion or had a lesion in the mesopallium dorsal to LMAN. (B) MMAN-lesioned + deafened birds ($n = 6$) and MMAN-intact birds ($n = 3$). For both A and B, the percent change in entropy variance (*y*-axis) is relative to the values of each bird 14 days earlier before deafening but after LMAN lesions; ~0% means that no change occurred as a result of deafening. The percent lesion size (*x*-axis) was calculated based on Nissl stain and singing-driven gene expression, as described in Materials and methods. Open circles, intact birds; closed circles, lesioned birds. *r* and *P* values were calculated from regression analyses.

MMAN did not prevent changes in either the syllable acoustic structure or song bout sequencing.

Discussion

The most salient findings of this study are that deafening-induced changes in syllable spectral structure and song syllable sequence occur on different time-courses, the sequence changes include global song bout reorganization, and there is a differential requirement of the anterior vocal pathway, i.e. the pallial-basal-ganglia-thalamic loop, for the syllable structure changes in song. Our study also provides more information on the mechanics of the changes and a quantitative means of detecting them. These findings and approach can be used for molecular and physiological studies on the early stages of deafening-induced vocal deterioration in the commonly studied closed-ended vocal learner, the zebra finch. Below, we discuss the types of changes found and the role of the anterior vocal pathway.

Deafening-induced changes

Previous studies on auditory feedback in deafened Bengalese finches, which sing a more variable song than zebra finches, have indicated that deafening can influence both song syllable spectral structure and sequences (Okanoya & Yamaguchi, 1997; Woolley & Rubel, 1997). However, motif and song bout sequence changes were not distinguished. In the most frequently studied species, zebra finches, previous studies focused on syllable spectral structure and motif sequence (Nordeen & Nordeen, 1992; Brainard & Doupe, 2000, 2001; Lombardino & Nottebohm, 2000; Sakata & Brainard, 2006) with little focus on song bout sequence. Here we investigated these questions.

For syllable structure, previous studies either measured gross syllable changes from spectrograms manually by eye or by digitized quantitative measure of the fundamental frequency and FM (Brainard & Doupe, 2000, 2001; Lombardino & Nottebohm, 2000). When using these methods, anecdotal changes in one or several birds were noted in the first 1–2 weeks following deafening but the first signs of statistically significant syllable spectral changes were not detected

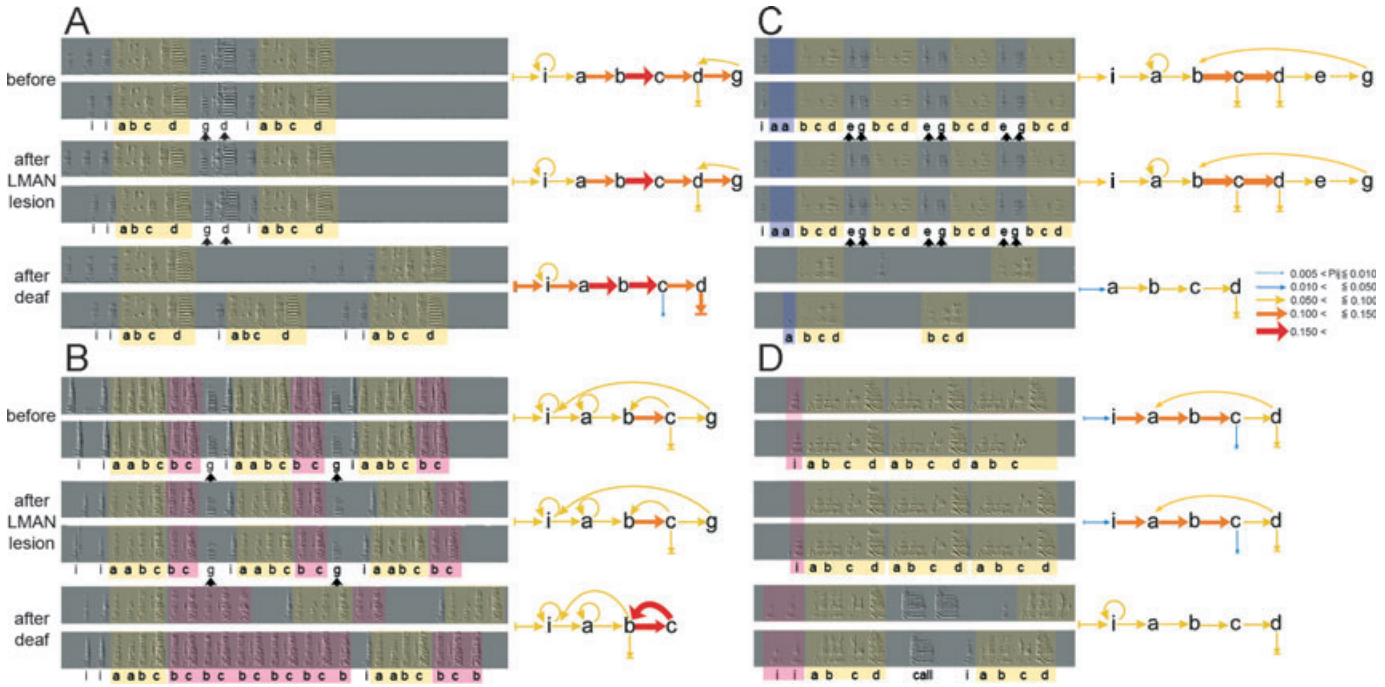

FIG. 10. Examples of song bout sequence changes after LMAN or MMAN lesion + deafening. In all panels, two example spectrograms are shown at 3 days before the lesions (top), 3 days after LMAN or MMAN lesions (middle) and 14 days after deafening (bottom). (A) Example LMAN-lesioned bird with dropped glue (g) and adjacent (d) syllables (black arrowheads) after deafening. This is the only example that we have seen where the dropped adjacent syllable occurs after the glue syllable. (B) Example LMAN-lesioned bird that, in addition to dropping of the glue syllable, increased repetition of previously repetitive syllables (b and c). (C) Example MMAN-lesioned bird where the glue (g) and adjacent (e) syllables were dropped, and the repetition of a previously repetitive syllable (a) was decreased after deafening. (D) Example MMAN-lesioned bird without a glue syllable that showed increased repetition of introductory syllables at the beginning of song bouts and calls at the end, as well as the usual production of fewer motifs per bout. Explanation of color coding and of transition diagrams to the left is the same as in the legend of Fig. 5. A, $n = 31$; B, $n = 35$; C, $n = 33$; D, $n = 33$ syllables.

until after 3–4 weeks in young adult deafened animals. We found that quantitatively assessed syllable structure changes were detected within 3 days of deafening. Further, the most robust feature of early changes in syllable spectral structure was syllable entropy. An even more consistent measure than entropy was a decrease in the entropy variance within syllables. Taken together, these results suggest that the first signs of deafening-induced changes in zebra finch song are an increase in syllable noisiness, associated with a more pronounced decrease in the variation in the spectral structure across a syllable. We do not know if this type of change will be detectable in the many other songbird species that, unlike the zebra finch or humans, produce more whistle-like syllables. Theoretically, entropy analysis is useful for syllables with a great amount of structure and weak for syllables with simple, especially tonal, structure but this remains to be tested.

The anecdotal changes within the first 1–2 weeks observed in the previous reports included a gross change to syllable 'clarity' by 2 weeks post-deafening (Nordeen & Nordeen, 1992), an apparent visible deterioration in syllable structure in several young birds at 1 week post-deafening (Lombardino & Nottebohm, 2000), and one bird with a repetitive increase in 'inter-motif syllable' production by 1 week (Brainard & Doupe, 2001). Our current results are compatible with these previous findings but are the first to quantify them and other early changes and to do so in a time-course analysis.

For the deafening-induced changes in song bout sequence, the most typical change was a decrease in the number of motifs per song bout. This change contributed to simplified song sequences. In contrast, in Bengalese finches, deafening has been noted to cause both simplification due to stuttering-like repetition of motifs and diversification in motif transitions (Okanoya & Yamaguchi, 1997). As Bengalese finch song has multiple motifs in a song bout, unlike zebra finch song that has only one motif, we believe that the diversification in Bengalese finches may reflect new transitions between relatively stable motifs.

The second most typical change was dropping of syllables that occur between motifs, whereas the motif sequence was hardly affected in the first 2 weeks after deafening. The most commonly dropped syllable was the glue syllable, which 14 of 29 (~50%) zebra finches produced in their song. These syllables had a harmonic stack structure with less FM relative to all other syllables. Syllables with similar acoustic characteristics have been called 'stack' syllables and are found in 80% of wild zebra finch males, constituting 17% of song elements (Zann, 1993, 1996). We noted that, in the sonograms of the wild birds, these stack syllables exist between and following motif sequences (Zann, 1993, 1996). Such syllables between motifs have also been called 'connecting' syllables (Yu & Margoliash, 1996), although their acoustic features and frequency of production were not defined. As such stack syllables have a similar acoustic structure to stack calls and such calls are incorporated into learned song (Zann, 1993, 1996), it is possible that there is a connection between when a syllable is incorporated into song, the level of entropy and the sensitivity to deafening. We suggest that the most frequently produced sequence of syllables, the motif, is the one that is most resistant to deafening-induced song deterioration and the less frequently produced glue syllable is less resistant. Such a mechanism would be consistent with the finding that age and song deterioration are negatively correlated (Lombardino & Nottebohm, 2000; Brainard & Doupe, 2001). In the 2 week period examined, we did not observe insertion of syllables except for calls at the end of song bouts. Thus, our results

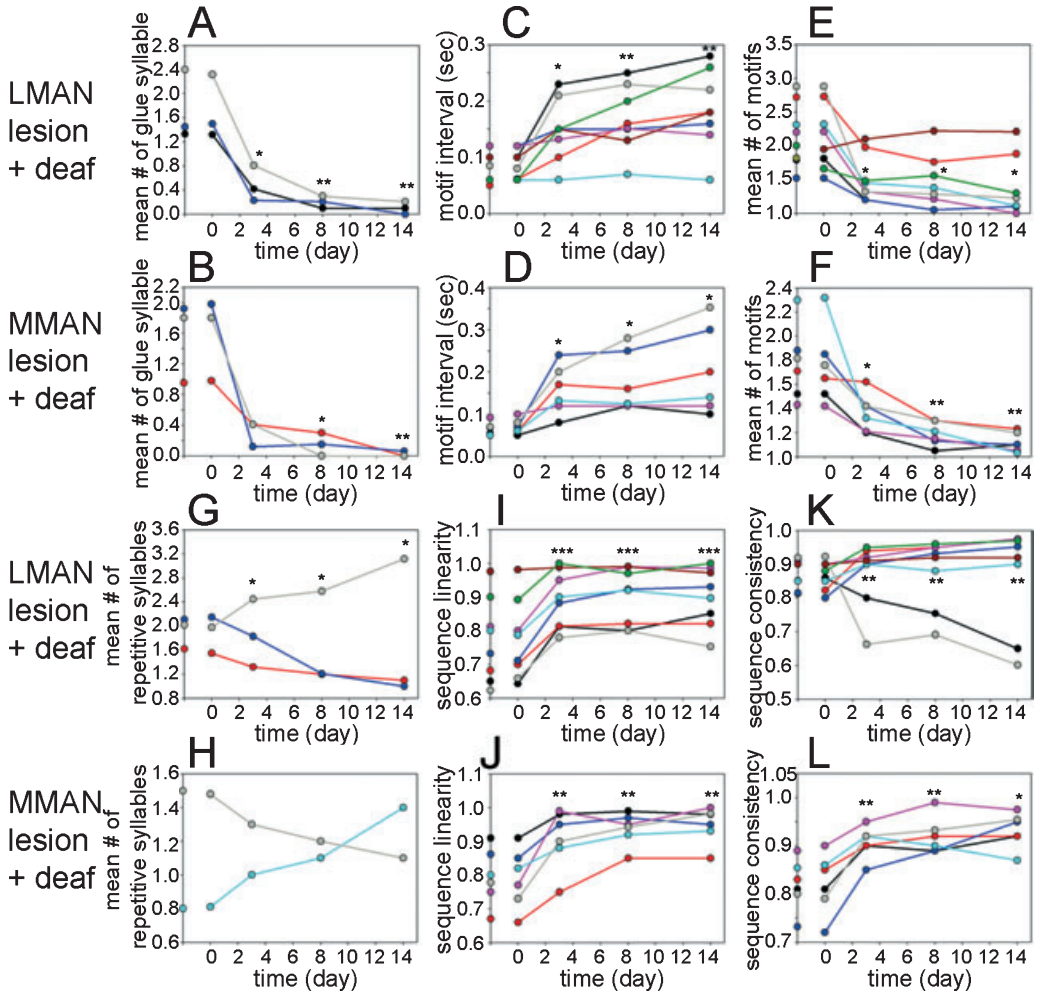

FIG. 11. Quantification and time-course analysis of changes in song bout sequencing in LMAN- and MMAN-lesioned + deafened birds. Graphs for six of the seven song bout sequence changes in deafened LMAN- (A, C, E, G, I and K) and MMAN- (B, D, F, H, J and L) lesioned birds. Colors indicate the same bird for LMAN- or MMAN-lesioned animals. For each graph, the value before day 0 is 3–5 days before the lesions, the value at 0 is 3–5 days after the lesions and the values thereafter begin after the birds were deafened. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, paired *t*-test, $n = 30$ song bouts per bird at each time-point. Explanation of statistical values is the same as that in the legend of Fig. 6A.

suggest that syllable dropping is more likely than syllable addition due to deafening.

Other types of changes that occurred were increased or decreased repetition of syllables that were repeated before deafening or repetition of call-like syllables that were introduced at the end of song bouts. The increased repetition sounded as if the birds stuttered. Although the term 'stuttering' has been used for both songbirds (Okanoya & Yamaguchi, 1997; Leonardo & Konishi, 1999; Helekar *et al.*, 2000; Cooper & Goller, 2004) and humans (Buchel & Sommer, 2004; Ward, 2006; Yairi, 2007), we do not know if this stuttering is similar in both, which in humans can occur for syllables, words and whole phrases. In humans, becoming deaf in adulthood can alleviate stuttering, indicating that human stuttering requires auditory feedback (Ward, 2006). In contrast, in zebra finches, our results indicate that stuttering can be induced by the absence of auditory feedback. The stuttering that we observed in the finches had a different time-course of onset relative to changes in dropped syllables and number of motifs per bout. This suggests that different mechanisms underlie the production of syllable repetition, motif repetition and inter-motif syllables. The fact that deafening also induces stuttering in Bengalese finches (Okanoya & Yamaguchi, 1997) suggests a shared trait of this deafening-induced change at least among these finches; however, in Bengalese finches it is not clear whether some of these syllables were produced repetitively before deafening. In terms of mechanisms, the differences in the rates of syllable structure and sequence changes prompted us to examine possible neural mechanisms for these changes.

Role of anterior vocal pathway

The LMAN and MMAN are pallial nuclei of pallial-basal-ganglia-thalamic-loops (Doupe *et al.*, 2005; Jarvis *et al.*, 2005; Kubikova *et al.*, 2007) where the striatal part consists of Area X and the thalamic parts are the dorsal lateral nucleus of the dorsomedial thalamus and dorsomedial nucleus of the posterior thalamus, respectively, in the dorsal thalamus (Fig. 1). When LMAN and MMAN are lesioned, they disrupt the output of these loops onto neurons of the motor pathway. The output from LMAN is onto RA and that from MMAN is onto HVC (Fig. 1) (Foster & Bottjer, 1998, 2001; Kubikova *et al.*, 2007). RA has been proposed to control song syllable structure and HVC to control song sequencing (Yu & Margoliash, 1996). In this regard, lesions to LMAN would be predicted to prevent song syllable

structure changes, whereas lesions to MMAN would be predicted to prevent song sequence changes. Previous studies have found that lesions to LMAN prevent deafening-induced changes in song syllable structure as well as changes in motif sequencing in zebra finches (Brainard & Doupe, 2000, 2001) but song bout sequence had not been quantified.

In the current study, we found that lesions to LMAN prevented changes to the syllable structure but had no effect on changes of song bout sequencing, consistent with the hypothesis. However, lesions to MMAN did not affect deafening-induced changes in syllable structure or song bout sequencing, inconsistent with the hypothesis. These findings lead to a modification of the hypothesis, this being that LMAN has a role in causing song syllable changes and motif sequence changes, perhaps via RA, but another part of the system may control the song bout sequence changes. Other candidates include the nucleus interface of the nidopallium and nucleus uvaeformis, both of which project to HVC (Fig. 1) (Fortune & Margoliash, 1995; Wild *et al.*, 2000). Nucleus interface of the nidopallium lesions affect song sequencing in Bengalese finches by inducing fewer motif transitions within song (Hosino & Okanoya, 2000). However, nucleus interface of the nidopallium lesions in zebra finches do not affect motif sequence production (Cardin *et al.*, 2005), although song bout sequence and age were not examined. Nucleus uvaeformis lesions, like lesions to HVC itself, lead to a dramatic loss in the ability to sing normal song; the birds only produce a song with one or several degraded syllables (Coleman & Vu, 2005). However, if unilateral nucleus uvaeformis lesions are made, the bird's singing behavior can recover and the recovered song has subtle changes in song motif and song bout sequences (Coleman & Vu, 2005). Taken together, these results suggest that both nucleus interface of the nidopallium and nucleus uvaeformis song nuclei may be good candidates for actively changing song bout sequence via HVC following deafening. Other possibilities are that MMAN has a role in song bout sequence in other species or that the deafening-induced changes in song bout sequence are a passive response to deafening instead of an active process controlled by a brain nucleus or system.

Interestingly, we did not detect short-term (3–5 days) changes in syllable entropy, song bout or motif sequence due to LMAN or MMAN lesions before deafening. Although this was not our focus, previous studies on hearing in intact animals have noted changes of increased stereotypy in syllable fundamental frequency at 1–3 days following LMAN lesions (Kao *et al.*, 2005; Kao & Brainard, 2006) and decreased stereotypy of song motif and bout sequence linearity at > 6 days after MMAN lesions (Foster & Bottjer, 2001). These findings and others have been used to support the idea that the anterior vocal pathway in adults is involved in vocal practice or exploration (Jarvis *et al.*, 1998; Kao *et al.*, 2005; Olveczky *et al.*, 2005; Kao & Brainard, 2006). We offer several possible explanations for the differences between studies: (i) for MMAN, we assayed for changes only within 3–5 days before deafening but the MMAN-induced effects could have shown up later after more singing [the earlier study examined quantitative changes only after 1 month (Foster & Bottjer, 2001)]; (ii) after LMAN lesions, the fundamental frequency may become stereotyped rapidly but the entropy variance may not change; or (iii) the effects of deafening may overshadow or over-ride the effects of MMAN and LMAN lesions. For the latter possibility, it is possible that the LMAN lesions alone would lead to long-term increases in entropy stereotypy in the absence of deafening and thereby over-ride deafening-induced changes; for MMAN lesions, deafening could have over-ridden possible long-term effects on song bout sequencing, as most birds showed an increase in linearity instead of a decrease. Finally, although MMAN does not appear to be required for deafening-induced sequence plasticity, it could still play a role in syllable sequencing during normal hearing. Overall, given our findings, these hypotheses are testable and should lead to further insight into the brain mechanisms of deafening-induced deterioration with parallels to human speech.

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Abbreviations

FM, frequency modulation; HVC, nucleus HVC; IEG, immediate-early gene; LMAN, lateral magnocellular nucleus of the anterior nidopallium; MMAN, medial magnocellular nucleus of the anterior nidopallium; RA, robust nucleus of the arcopallium.

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