

# Adult Bengalese Finches (*Lonchura striata* var. *domestica*) Require Real-Time Auditory Feedback to Produce Normal Song Syntax

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**ABSTRACT:** Songbirds develop their songs by imitating songs of adults. For song learning to proceed normally, the bird's hearing must remain intact throughout the song development process. In many species, song learning takes place during one period early in life, and no more new song elements are learned thereafter. In these so-called close-ended learners, it has long been assumed that once song development is complete, audition is no longer necessary to maintain the motor patterns of full song. However, many of these close-ended learners maintain plasticity in overall song organization; the number and the sequence of song elements included in a song of an individual vary from one utterance to another, although no new song elements are added or lost in adulthood. It is conceivable that these species rely on continued

auditory feedback to produce normal song syntax. The Bengalese finch is a close-ended learner that produces considerably variable songs as an adult. In the present study, we found that Bengalese finches require real-time auditory feedback for motor control even after song learning is complete; deafening adult finches resulted in development of abnormal song syntax in as little as 5 days. We also found that there was considerable individual variation in the degree of song deterioration after deafening. The neural mechanisms underlying adult song production in different species of songbirds may be more diverse than has been traditionally considered. © 1997 John Wiley & Sons, Inc. *J Neurobiol* 33: 343–356, 1997

**Keywords:** auditory feedback; vocal motor control; *Lonchura striata*; close-ended learner; deafening

## INTRODUCTION

Songbirds develop their songs by imitating adults (reviewed by Kroodsma, 1982), a process often compared to human speech acquisition. In the pro-

cess of song learning, it is critical that a bird have intact hearing for normal song development; deafening a bird prior to the completion of song learning (crystallization) results in the development of abnormal songs (Konishi, 1964, 1965; Nottebohm, 1968; Marler and Waser, 1977; Marler and Sherman, 1983).

In contrast to the absolute importance of the auditory experience to the song-learning process in all oscine species examined to date (Kroodsma and Baylis, 1982), the importance of audition to song production in adulthood varies among different species. Canaries rely on continuous auditory

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feedback to produce song; songs of one bilaterally deafened adult canary deteriorated in 7 days, and within 13 months (Nottebohm et al., 1976) they became very similar to songs of neonatally deafened birds (Guettinger, 1981). In contrast, white-crowned sparrows and chaffinches do not need auditory feedback to produce song in adulthood; deafened adults of these two species retained their normal song for as long as 2 years after the operation (Konishi, 1965, Konishi and Nottebohm, 1969). Zebra finches also do not require immediate auditory feedback to maintain their learned song (Price 1979, Bottjer and Arnold, 1984), but intact hearing is necessary for the long-term maintenance (<6 weeks) of adult song (Nordeen and Nordeen, 1992).

Traditionally, the audition-dependent song production system seen in canaries has been explained as a function of its distinct pattern of song learning (Nottebohm et al., 1976). The canary is an open-ended learner in which song learning takes place not only during their first year of its life, but also in adulthood, so that new song elements are replaced with some of older ones every year (Nottebohm and Nottebohm, 1978, 1986; Guettinger, 1979; but see Hartly et al., 1997). Because of this extended ability to learn songs in adulthood, it was reasoned that continuous auditory input is necessary to produce normal songs in adult open-ended learners (Nottebohm et al., 1976). The other three species mentioned above are close-ended learners in which song learning takes place only once during a restricted period early in life, after which no further song elements are learned. In these species, it has been assumed that auditory memory formed during the sensitive phase is transformed into static motor memory in adulthood such that little auditory feedback is necessary for the expression of vocal motor patterns. However, this view was proposed based on results obtained from a limited number of species. It is possible that factors other than different patterns of song learning may dictate the role played by auditory feedback in song production among different species of passerines.

Canaries differ from other species not only in their manner of song learning, but also in their song patterns. Canaries belong to a group of birds that produce variable songs; the number and the sequence of song elements included in songs vary every time a bird sings. In contrast, songs of white-crowned sparrows, chaffinches, and zebra finches are short, discrete, and highly stereotyped such that the individual songs sung by an adult are virtually

identical (although songs sung by different individuals are different). It is conceivable that real-time auditory feedback might play a crucial role in indexing different song elements in variant singers such as canaries, but not in stereotyped singers such as white-crowned sparrows. This led us to hypothesize that canaries rely on continuous auditory feedback not only because they are open-ended learners, but also because they need to produce variable songs. To examine the relationship between song variability and audition dependency of song production, it is important to test the role of audition in a species that produces variable songs, yet is a close-ended learner.

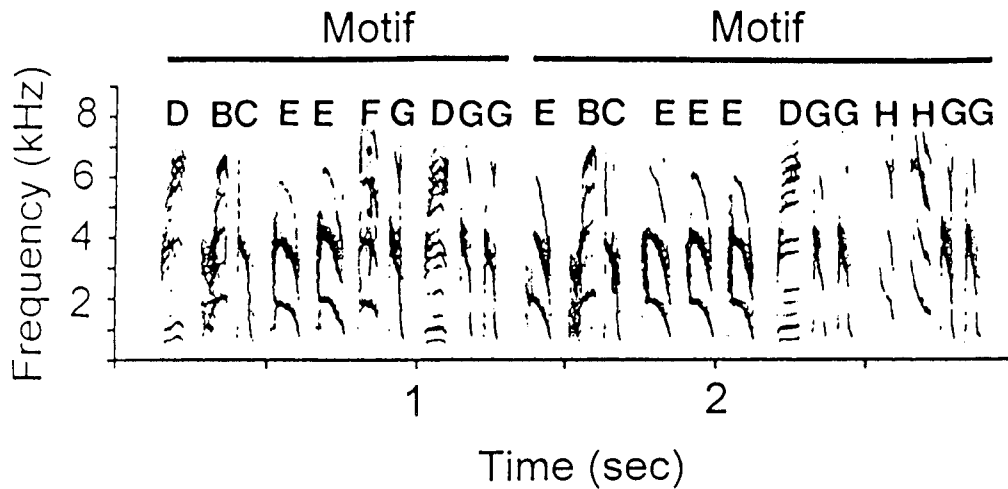
In our present study, we examined the importance of audition for song production in Bengalese finches (*Lonchura striata* var. *domestica*), a close-ended learner with variable songs, by deafening adults and observing the subsequent changes in their songs. The close-endedness of the Bengalese finches was determined by several kinds of experiments. Clayton (1987) used time-limited tutoring techniques to determine sensitive period of song learning to be between 35 and 70 days of age. Okanoya (personal observation) isolated juvenile male Bengalese finches from conspecific males until 90 days of age and showed that the finches failed to develop normal adult songs. Immelmann (1969) described a normal time course of song development in Bengalese finches. Taken together, after 90 days of age, the song elements that make up the songs of an individual will not be added or lost and the acoustic morphology of these elements remains unchanged throughout the life of the individual.

We report here that when adult Bengalese finches were deafened, the temporal structure of songs deteriorated in as little as 5 days, while morphology of song elements degenerated more slowly. This result is consistent with the hypothesis that some passerines use real-time auditory feedback in adulthood to produce song variation regardless of their pattern of song learning. A part of this study has been published in abstract form elsewhere (Okanoya et al., 1990, 1991; Okanoya, 1996). Similar results were recently reported from other laboratories (Watanabe and Aoki, 1996; Wooley and Rubel, 1996).

## MATERIALS AND METHODS

### Subjects and Surgery

Birds were obtained from local pet suppliers and kept in aviaries under a fixed 14:10 h light/dark cycle. The



**Figure 1** Part of a song bout obtained from a control bird illustrating song notes, motif, and how note sequence could vary from one motif to another. The first motif began by note D, while the second was initiated by note E. Note E was repeated twice in the first motif but was repeated three times in the second; note F following note E in the first motif was replaced by note D in the second. Note D preceding the last two Gs in the first motif was replaced by repetition of note H.

ambient temperature of the aviary was maintained at  $\sim 24^{\circ}\text{C}$  and relative humidity was  $\sim 60\%$ . A total of nine male Bengalese finches, 5–24 months old, were used in this experiment. Song development is complete at approximately 90 days in Bengalese finches (Clayton, 1987; Okanoya, personal observation). Thus, all the birds were well beyond the age of song crystallization. Six finches were deafened by bilateral removal of the cochleae, following the procedure described by Konishi (1965) under Nembutal anesthesia (concentration = 0.012 mL/bird). Three birds were unilaterally deafened as a control. The removed cochleae were examined under a dissecting microscope to confirm that deafening was complete.

### Description of Song Structure

A birdsong is a discrete utterance, typically consisting of a series of notes separated from others by silent intervals. A note is considered here to be a single vocalization that is expressed as a continuous trace on a spectrogram. The song of Bengalese finches is less discrete than that of many other oscine species (Fig. 1). Bengalese songs are long bouts of vocalizations (up to 30 s) that contain a series of notes. Within a bout, a motif of note sequences is repeated multiple times. The number and the types of notes included in a motif can change from one utterance to another (average 11.1 notes in a motif, range 7–17). However, notes within a motif are not ordered at random;

a general sequence of notes can be recognized by sampling several motifs from an individual [Fig. 1; see also Figs 5(a) and 6(a)].

### Song Recording

For each recording session, at least three song bouts (average 4.5, range 3–9) were recorded from each bird. The result was an average of 215.1 notes (range 99–481), 21 motifs (range 14.5–39.5) recorded from each individual during each recording session. For five birds (four deaf and one control), recordings were made in a quiet room using a directional condenser microphone (Sony ECM-K7) and a cassette tape recorder (Sony TCM-18). Recordings for the remaining four birds (two deaf and two control) were made in a soundproof room with a condenser microphone (Aiwa CM-S3) attached to a digital audio tape recorder (Aiwa HD-S1). Further analyses were conducted on recordings made on the same settings.

The birds were induced to sing “undirected” song (the song sung by itself and not addressed to females) (Sossinka and Bohner, 1980) by isolating them from others in the recording room. We chose to use undirected rather than directed song because the duration of the song bouts tended to be longer in the former case; thus, a larger number of motifs could be sampled in a given recording session. Recordings were made on 30 days before the surgery (day -30), on the day of the surgery (immediately before the operation, day 0), and 5 (day 5), 30

(day 30), and 60 days (day 60) after the surgery for both control and deafened birds.

## Analyses

**Number of Notes Lost.** The number of note types included in song bouts were compared before and after the surgery to examine if the variation of note types that make up song bouts change as a result of bilateral deafening. First, the number of note types found on day -30 and day 0 in all birds (both control and deaf) was compared to confirm that note types were not added or lost in songs of adult Bengalese finches, using the Mann-Whitney *U* test. Then, the number of note types found on day 0 were compared to those found on days 5, 30, and 60 in deaf birds using the Wilcoxon signed rank test.

**Phonological Analysis.** Note phonology, or the acoustic morphology of notes, was examined pre- and postoperatively using sound spectrograms (SIGNAL Sound Analysis System, Engineering Design) (Beeman, 1992). Each unique note found in songs recorded on day 0 was alphabetically labeled for each individual. We used a spectrogram cross-correlation technique (the CORMAT program supplied in SIGNAL version 2.0) (Beeman, 1992) to evaluate morphologic changes in songs (Nelson and Marler, 1993). The cross-correlation coefficient is an objective way to evaluate the similarity between two sounds (Clark et al., 1987). We correlated same notes and different notes emitted by an individual and found that same notes correlated better than different notes. The coefficient ranges from zero to one; the larger the coefficient, more similar the two sounds are. One representative song motif was randomly selected from each recording session, and note-by-note similarities between note types were calculated.

To confirm that note phonology does not change over time in adult Bengalese finches after song crystallization, the cross-correlation coefficients calculated between notes recorded on day -30 and day 0 were compared to the coefficients obtained between notes emitted within day 0 in all nine birds using the Wilcoxon signed rank test. If the subjects used in the present experiment had not crystallized their songs, one would have expected that a pair of notes emitted on separate dates would be less similar than a pair of notes produced on a same day.

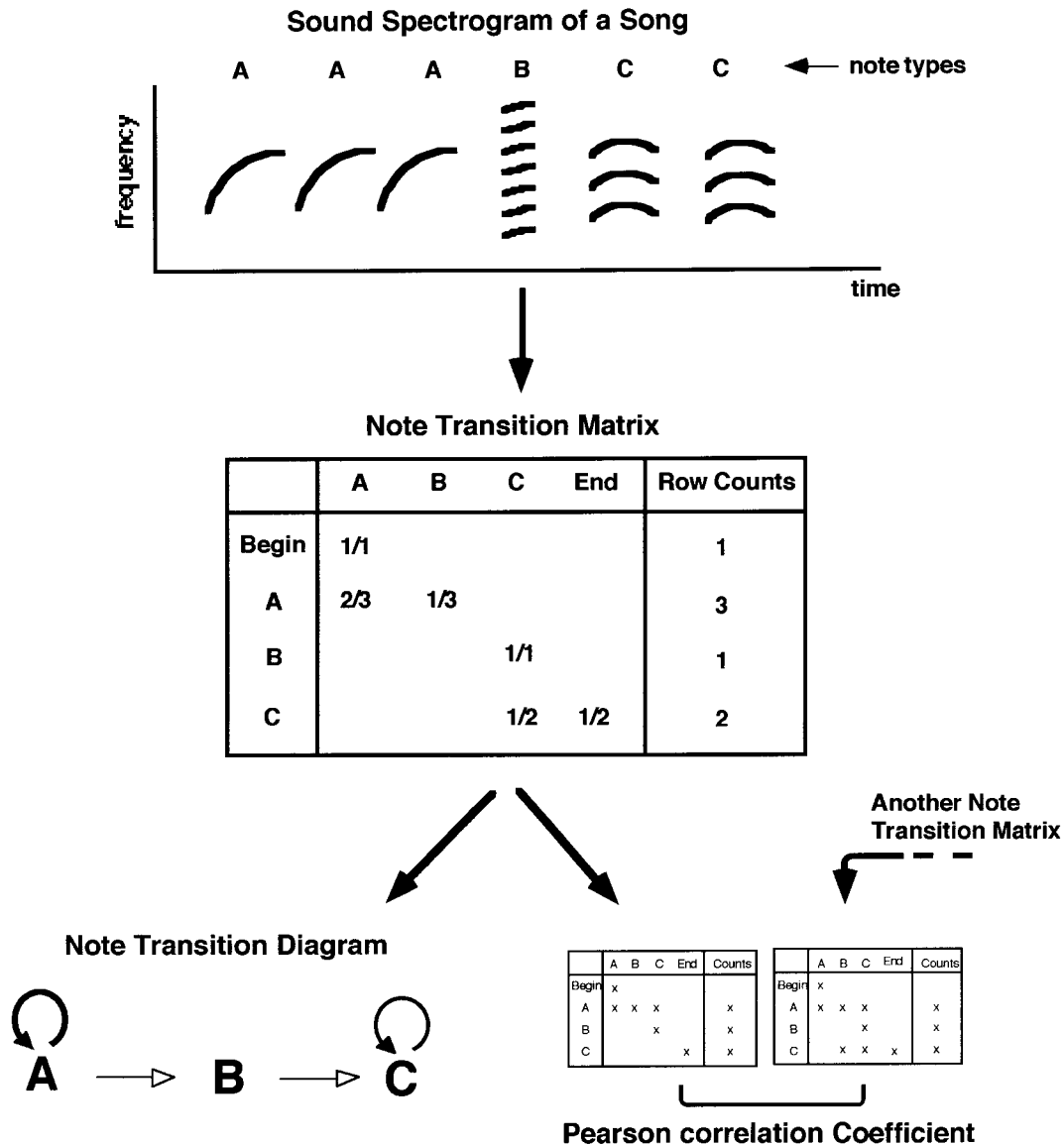
To determine whether note phonology became more or less variable after bilateral deafening, correlation coefficients calculated between pairs of notes recorded on day 0 were compared to coefficients calculated between pairs of notes recorded on day 60 in all deaf birds using the Wilcoxon signed rank test. If the phonological variation either increased or decreased after surgery, coefficients obtained from notes recorded on day 60 were expected

to be significantly different from coefficients calculated between notes recorded on day 0.

To determine whether preoperative note phonology changed postoperatively, the cross-correlation calculated between notes recorded on day 0 and those recorded on days 5, 30, and 60 (which indicate the structural similarity of notes emitted before and after the surgery) were compared to correlation between notes recorded on day 0 and day -30 (which indicate the phonological similarity of notes emitted in two separate dates before the surgery) in all deaf birds, using the Wilcoxon signed rank test. Some subjects lost preoperative note types after the operation. Only the notes that could be identified on the later recordings of the deaf birds (average 6.1, range 4-12) were used for the analysis of phonological consistency.

**Acoustic Properties of Lost or Retained Song Elements.** Deafening resulted in the loss of some song notes. Is there a systematic difference in the acoustic structure of note types such that some can be produced without auditory feedback while others require intact audition? To answer this question, the acoustic properties of lost and retained elements were examined. Retained note types were defined as all the note types found in recordings made on day 60, and lost note types were defined as the preoperative notes that could not be found day 60 recordings. One exemplar from each note type was sampled from songs of each deaf bird on day 0, and the following five acoustic properties were measured: (a) dominant frequency (DF), (b) fundamental frequency (FO), (c) frequency range (FR), (d) bandwidths measured at 10 dB downpoint from DF (BW), and (e) duration. These parameters were previously shown to be biologically significant to Bengalese finches when they acoustically discriminate vocalizations of males and females (Okanoya and Kimura, 1993). The first four frequency parameters were measured using the frequency spectrum calculated from each note type, and the duration was measured from a sound spectrogram obtained on a Kay 5500 DSP Sona-graph with a 300-Hz analysis bandwidth. Student *t* test was used to examine if the two groups of notes differed in any of the acoustic parameters. Multivariate analysis of variance (MANOVA) was used to test if retained and lost notes differed in measured parameters.

**Sequence Analysis.** To estimate the variability of note sequences within bouts of singing, we constructed a transition probability matrix for each recording session. Briefly, the matrix was calculated by counting the number of times a given note was followed by all the note types in all song bouts recorded on a given recording date, and the figures were divided by the total number of occurrences of a given note recorded on that day (Fig. 2). All note types obtained up to day 30 were matched with preoperative note types. However, the sequence analysis



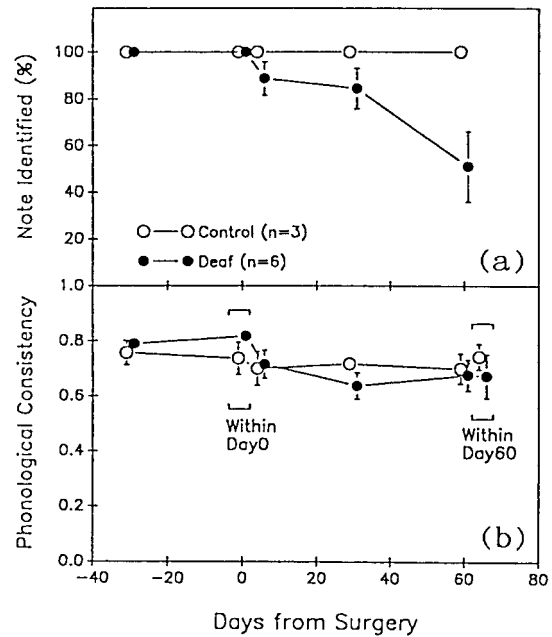
**Figure 2** Method used to build both note transition matrices and transition diagram, and to calculate the Pearson correlation coefficient that indicates note sequence consistency. (Top) The sound spectrogram of a simplified song bout. Note that each note type is labeled with a unique letter. Based on this spectrogram, a note transition matrix (second diagram from the top) was constructed. In this example, the bout began with an A, so that count 1 was entered into the cell “Begin–A (row by column).” Then, note A was followed by two As and a single B, so that count 2 was entered into the cell “A–A” and count 1 into the cell “A–B.” Each count was then divided by a sum of row counts. Based on this transition matrix, a transition diagram of the song bout was drawn (bottom left). Note that transitions that took place with higher probability are connected by thick arrows, while transitions that rarely took place are connected by thin arrows. This transition matrix was also used to calculate the Pearson correlation coefficient that quantifies how consistent or variable note sequences are. Two transition matrices were compared (bottom right diagram), and corresponding elements that were not zeros were used to calculate the Pearson correlation coefficient.

could not be carried out on song bouts recorded on day 60 because many of the note types emitted were too dissimilar from those found preoperatively to be identified.

Transition diagrams based on transition matrices visually illustrate the degree of note sequence stereotypy [see Figs 5(b) and 6(b)]. If note sequences within motifs are produced in a highly stereotyped manner, the transition diagram consists of a set of alphabets (denoting note types) that are connected by thick arrows, indicating that one note was followed by another with high probability. In contrast, if note sequences of motifs are very variable within a song bout of an individual, the transition diagram would contain a set of alphabets connected with many thin arrows.

To quantitatively assess the changes in note sequence before and after the surgery, we calculated Pearson correlation coefficients for the transition matrices. We first determined whether the note sequence that makes up a motif became more variable after deafening. Song bouts recorded from an individual on a given day were divided into two groups, a transition probability matrix was obtained from each group, and correlation coefficients between the two were then calculated. If the coefficient was high, a bird repeated motifs that were stereotyped in note sequence within a day. If the coefficient was low, the note sequence included in a motif changed considerably every time a bird sang within a day (within-day data). By comparing within-day coefficients calculated before (on day 0) and after surgery (on day 60) in each deaf individual using the Wilcoxon signed rank test, we determined whether the magnitude of sequence variation changed after deafening. We next tested whether the preoperative note sequence was altered after the surgery by comparing pre- and postoperative note sequences. Despite some variation of the note sequence, a prototypical note sequence included in a song motif could be identified from the song bouts of each individual (as shown in transition diagrams). Pearson correlation coefficients calculated between pre- and postoperational matrices (day 0 against days 5 and 30) were compared to coefficients calculated between matrices obtained on two preoperational dates (day -30 and day 0) of deaf birds, using the Wilcoxon signed rank test. If the coefficients between the pre- and postoperative matrices significantly differed from the coefficients between two preoperative matrices, then we concluded that the note sequence changed after the surgery.

All statistical tests were two-tailed and run at  $p < 0.05$  levels of significance. When there were multiple number of comparisons (day 0 vs. day 5, day 0 vs. day 30, etc.), the family confidence interval for the two or three comparisons was set at 90% with a sequential Bonferroni adjustment for each variable (i.e., significant alpha values necessary to reject  $H_0$  were set at 0.05 and 0.1 for two comparisons, and 0.033, 0.05, and 0.1 for three comparisons for the most significant to the least signifi-



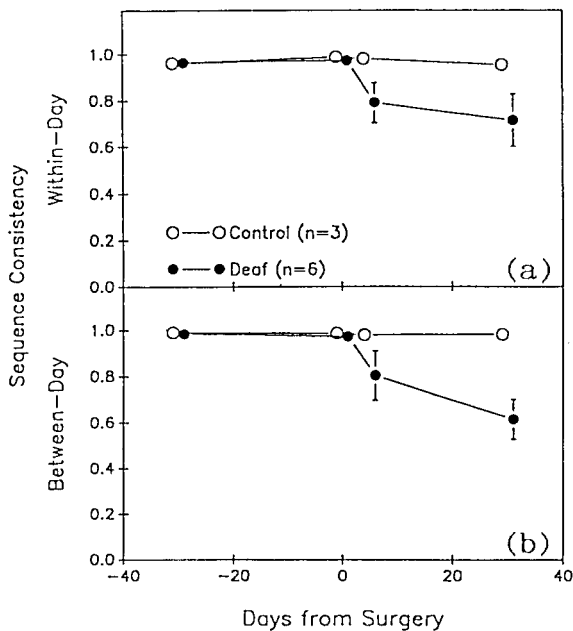
**Figure 3** (a) Number of note types identified in post-operative recordings, expressed as a percentage of the number of unique note types identified in preoperative recordings in three control (open symbol) and six deaf (closed symbol) birds. Error bars are  $\pm 1$  S.E. Overlapping symbols are displaced horizontally for clarity. (b) Phonological consistency of song notes on day 0 (in brackets) was used as a reference to compare the phonological consistency of the song before and after deafening. Phonological consistency was defined as the average of correlation coefficients obtained from the spectrotemporal matrices of corresponding notes. Symbols as in (a).

cant two/three comparisons) (Rice, 1989; Wright, 1992).

## RESULTS

### Songs of Bengalese Finches before the Surgery

In all nine birds, the number of note types found on day -30 and day 0 were identical [Fig. 3(a)]. Thus, none of the subjects was adding or omitting note types in its song when we carried out our experiment. Note phonology was considerably stereotyped before the surgery in all birds. Cross-correlation coefficients calculated between notes produced on day -30 and day 0 in all nine birds (average coefficient = 0.779) did not significantly differ from



**Figure 4** (a) Variations in song sequence (expressed as within-sequence consistency) among bouts of singing within each recording session. Sequence variation was defined as a correlation coefficient of the two transition matrices obtained from each recording session. Symbols as in Figure 3(a). (b) Sequence consistency of the song before and after deafening. Sequence consistency was defined as a correlation between the reference transition matrix and the transition matrix of each recording day. Symbols as in Figure 3(a).

coefficients calculated between pairs of notes produced within day 0 (within-day 0 average 0.802;  $Z = -0.652$ ,  $p = 0.515$ ) [Fig. 3(b)]. Thus, note phonology remained constant over time before the surgery in all birds.

Note sequences of all nine birds recorded on the day of the surgery were considerably stable (within-day 0 average = 0.977) [Fig. 4(a)] and did not differ from sequences produced on day -30 (between day -30 and day 0 average = 0.986;  $Z = -1.481$ ,  $p = 0.139$ ) [Fig. 4(b)].

Taken together, the number of note types included in song bouts remained constant over time, and both phonological and syntactical structures of songs were stable prior to deafening, all of which are indicative of crystallized songs. Control and deaf birds did not differ in the degree of phonological and syntactical consistency prior to the surgery (phonological consistency: day -30:  $U = 4$ ,  $p = 0.262$ ; day 0:  $U = 5$ ,  $p = 0.381$ ; sequence consis-

ency: day -30:  $U = 7$ ,  $p = 0.714$ ; day 0:  $U = 4$ ,  $p = 0.262$ ; day 0 and day -30:  $U = 5$ ,  $p = 0.381$ ).

### Number of Note Types after the Surgery

Deaf birds lost 51.5% of preoperational notes by 60 days after the surgery, while control birds retained all the preoperative notes [Fig. 3(a)]. This difference between the deaf and control groups on day 60 was statistically significant ( $U = 1.5$ ,  $p = 0.048$ ).

### Phonological Changes after the Surgery

Phonological consistency found within day 0 and day 60 in deaf birds did not significantly differ ( $Z = 1.753$ ;  $p = 0.0796$ ). Thus, the phonology of notes produced within a day became neither more variable nor more stereotyped after the surgery, at least in surviving note types that were found at day 60.

When the phonological structure of notes recorded before and after the surgery was examined in deaf birds, some deterioration was detected. Cross correlation-coefficients calculated between notes recorded on day 0 and day 30 differed significantly from coefficients calculated between notes recorded on day -30 and day 0 ( $Z = -2.201$ ;  $p = 0.0277$ ) [Fig. 3(b)]. This indicates that note phonology degenerated 30 days after surgery. However, the phonological degeneration was not detected on two other postoperative dates; coefficients calculated between notes emitted on day 0 against days 5 and 60 did not significantly differ from coefficients calculated between notes recorded before the surgery ( $Z = -1.572$ ,  $p = 0.1159$  for day 5;  $Z = -1.461$ ,  $p = 0.1441$  for day 60). This inconsistency in note deterioration is likely to be ascribed to our ability to identify note types. Among all the note types recorded postoperatively, we performed phonological analyses only on those notes that we could confidentially identify with the note types found preoperatively. We had no difficulty in matching pre- and postoperative note types up to day 30. However, at day 60, some notes degenerated to a point where we could not identify confidentially their preoperative counterparts. Thus, it is likely that we underestimated the degree of phonological deterioration, especially on the later dates after the operation.

**Table 1** Acoustical Parameters of Retained and Lost Note Types

Parameters	Retained Note Type ( <i>n</i> = 37)	Lost Note Type ( <i>n</i> = 22)	MANOVA		
			<i>F</i>	<i>df</i>	<i>p</i> Value
DF (Hz)	3810 ± 223	3203 ± 242	0.658	5, 53	0.657
F0 (Hz)	2076 ± 204	2067 ± 184			
FR (Hz)	951 ± 171	887 ± 161			
BW (Hz)	1282 ± 153	1236 ± 210			
Duration (ms)	72 ± 5	82 ± 7			

### Acoustic Characteristics of Lost and Retained Note Types

The acoustic properties measured did not differ between lost and retained note types (Table 1). Thus, there does not appear to be a systematic acoustic difference between note types that can be produced with or without auditory feedback.

### Song Sequence Deterioration after the Surgery

Compared to the preoperative note sequence variation, the postoperative sequence variation became larger in three deaf birds, while it remained constant in the remaining three deaf birds as well as in control birds. As a group, the postoperative note sequence of deaf birds songs at day 30 had a larger variance than before ( $Z = -1.992$ ;  $p = 0.0464$ ) but not on day 5 ( $Z = -1.782$ ;  $p = 0.0747$ ).

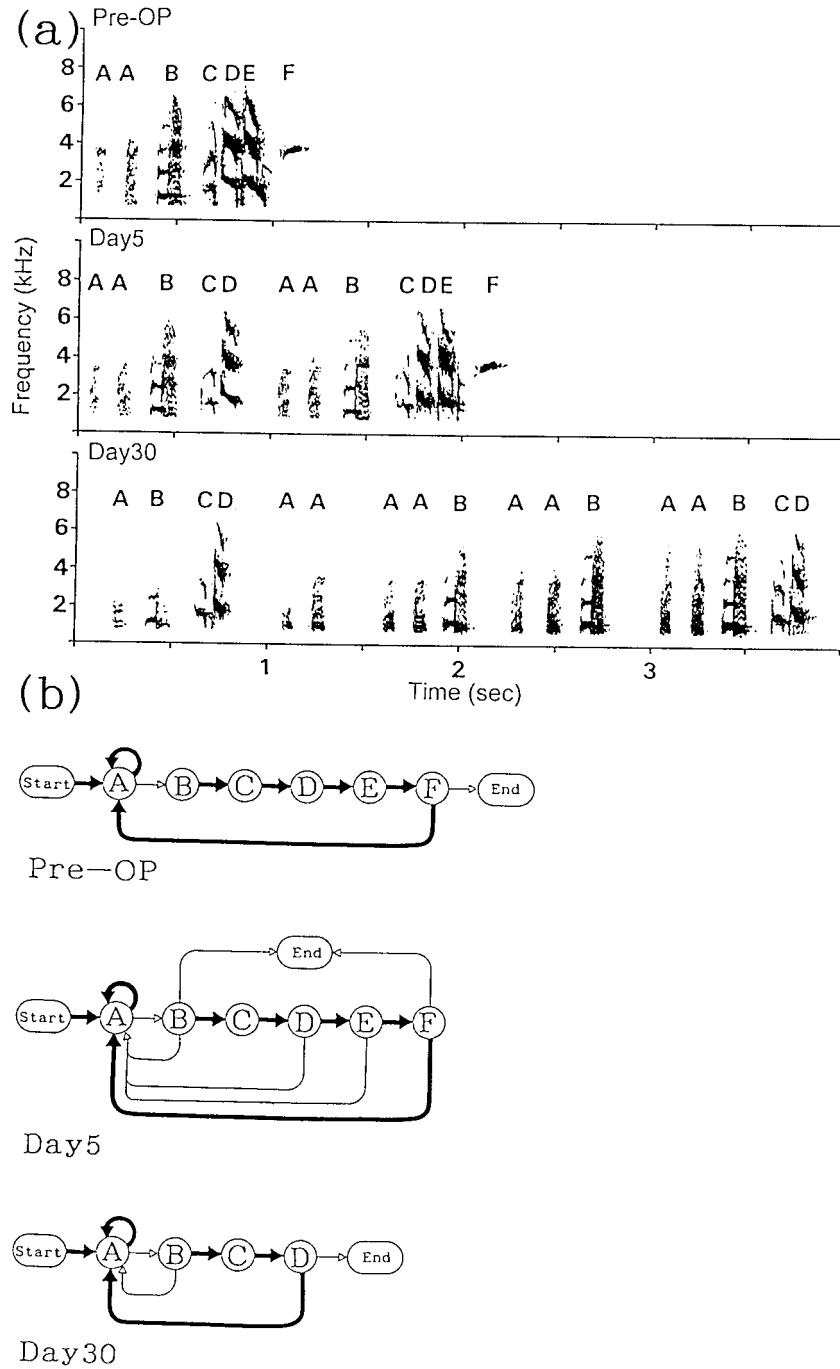
In addition to the increase in overall variation in note sequence, the prototypical note sequences that was present prior to the surgery were significantly altered in deaf birds but not in control birds (Fig. 4). When sequence consistency calculated between songs recorded on day 0 against days 5 and 30 were compared to consistency calculated between songs recorded on two preoperational dates in deaf birds, note sequences were significantly different on day 30 ( $Z = -2.429$ ;  $p = 0.0152$ ) but not on day 5 ( $Z = -1.896$ ;  $p = 0.0576$ ). Despite the statistical insignificance, the deterioration of note sequences was noticeable at postoperative day 5 in three of six deaf birds (Figs. 5 and 6).

In addition to the quantitative analysis of note sequence deterioration, a detailed description of the songs of deafened individuals illustrates the magnitude of deterioration. Five of six bilaterally deafened birds sang songs with abnormal note sequence within 30 days of surgery. Two different patterns of note sequence deterioration were ap-

parent: stuttering and scrambling. The day after the surgery, three birds were observed to sing stuttered songs in which a group of song notes was sung repeatedly before proceeding into the next part of the song. This type of stuttered note sequences was never observed prior to the surgery. An example of stuttered songs is shown in Figure 5(a). Before deafening, this bird sang a highly stereotyped song motif for a Bengalese finch [Fig. 5(a), top; and 5(b), top]. Five days after the surgery, however, many incomplete motifs that lacked the last few notes were repeated [Fig. 5(a), middle; and 5(b), middle]. Thirty days after the surgery, stuttering was profound and the bird failed to emit notes E and F, notes positioned at the end of its preoperative song [Figs. 5(a), bottom; and 5(b), bottom]. Scrambling, a type of song deterioration that resulted in a random note sequence, was observed in two other birds after surgery (e.g., Fig. 6). Figure 6(a) (top) shows a transition diagram from a bird which sang a complex but predictable song before surgery. At 5 days after surgery, however, some song bouts began with note B, which was never previously observed, and note G was often missing [Fig. 6(a), middle; and 6(b), middle]. At day 30, the order of notes included in the song became even more variable [Fig. 6(a), bottom; and 6(b), bottom] and showed an increased probability of novel note transitions that had not occurred before the surgery.

One remaining experimental bird did not change song sequence at all after the surgery up to day 30 (between day 0 and day 30 sequence consistency was 0.987). However, over the next 2 years, seven of 15 preoperative note types were lost such that the note sequence included in a motif was considerably altered, and the phonological structure of the remaining note types was significantly changed (phonological consistency

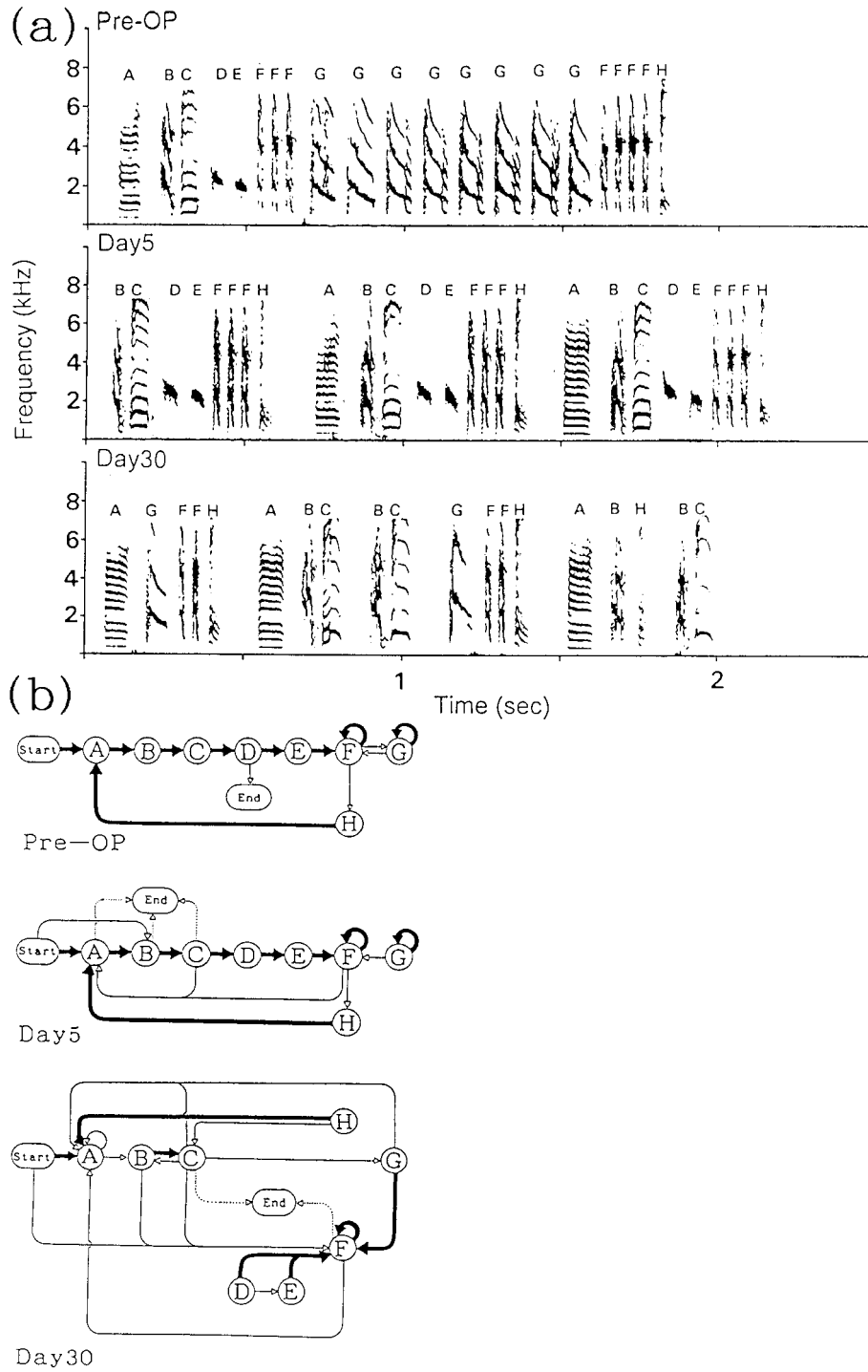




**Figure 5** Example of song stuttering after deafening. Upper figures (a) are the spectrograms of representative song sequences recorded just prior to the surgery and on postoperative days 5 and 30. Lower figures (b) are transition diagrams based on these recordings. Thick lines with closed arrowheads indicate transition probabilities > 50%. Thin lines with open arrowheads indicate transition probabilities > 10%.

within day 0 = average 0.736; phonological consistency between day 0 and year 2 = average 0.456;  $Z = -2.03$ ;  $p = 0.042$ , Wilcoxon signed

rank test). This magnitude of song alteration over the same period of time has not been observed in intact adult Bengalese finches (Okanoya, personal



**Figure 6** Example of song scrambling after deafening. Upper figures (a) are the spectrograms of a representative song sequence recorded just prior to surgery and on postoperative days 5 and 30. Lower figures (b) are transition diagrams based on these recordings. Thick and thin lines with open or closed arrowheads are as in captions in Figure 5. Dotted lines indicate that the transition from the last note to the end was <10%.

observation). Interestingly, the eight retained note types made up the early half of the original song motif. A postmortem examination of the cochlear nuclei of this subject (cytochrome oxydase histochemistry) (Brauth, 1990) confirmed that deafening had been complete: Both of the medullary cochlear nuclei had shrunk to 20% of their intact volume.

## DISCUSSION

In this study, we examined whether the Bengalese finch, a close-ended learner that produces considerably variable songs, relies on auditory feedback to produce songs in adulthood. We found that when adult Bengalese finches were deprived of auditory feedback by bilateral deafening, the temporal structure of their songs deteriorated shortly thereafter, and note phonology deteriorated more slowly. In addition, deaf birds eventually produced songs that include only half of the original note types. The song deterioration following bilateral deafening cannot be ascribed to the age of birds, because all birds used in this study were adults with fully crystallized songs, as seen in consistent syntax and phonology. Nor could it be a simple side effect of the surgery, because control birds that were unilaterally deafened using the same surgical procedure did not show deterioration in song structure. The rapid deterioration of song syntax indicates that adult Bengalese finches rely on moment-to-moment auditory feedback to produce the normal song syntax even after the song is fully crystallized. Proprioceptive feedback from the syrinx, the vocal organ, alone is apparently not sufficient to generate a normal syntax.

### Song Syntax Variation and Audition-Dependent Song Production

The rapid deterioration in song syntax observed in deafened adult Bengalese finches is similar to that observed in the adult-deafened canary. Nottebohm et al. (1976) reported that a deafened adult canary started singing shorter and more monotonous songs with fewer numbers of elements representing larger proportion of their songs within 7 days after surgery. Although syntactical analysis was not carried out in this canary study (nor in subsequent study carried out on the same bird) (Guettinger, 1981), it is plausible that the shorter song with fewer elements is a manifestation of song syntax deterioration. Together,

the results of Bengalese finch and canary are consistent with our hypothesis that passerines that produce variable songs require moment-to-moment auditory feedback in adulthood to produce normal song syntax regardless of their pattern of song learning. In these species, auditory feedback may play a dynamic role in indexing note syntax as a bird produces a song. Other species that possess song syntax variants (e.g., sedge warblers: Catchpole, 1976; European robins: Brindley, 1991; song sparrows: Stoddard et al., 1988; northern cardinals: Lemon and Chatfield, 1971; nightingales: Hultsch, 1993) would then be expected to show immediate deterioration in their syntax structure when deafened as adults.

Our attempt to explain the audition-dependent song production as a function of song variation in adult passerines is not mutually exclusive with the traditional view that open-endedness requires continued auditory feedback for song production in adulthood. In fact, the role of audition in open-ended, variable singers such as canary may be twofold: First, it produces syntax variation during song production phase; and second, it acquires new song elements during the song-learning phase. When an adult canary is deafened during the song production season, as was done by Nottebohm et al. (1976), the dual role of audition in adult canary may become apparent as a rapid reduction in note diversity and a drastic alteration of song structure that takes place within a year.

### Auditory Feedback and Note Phonology

The time course of phonological deterioration observed in deafened adult Bengalese finches is comparable to that observed in deafened adult zebra finches (Nordeen and Nordeen 1992). Thus, auditory feedback appears to play similar roles in a similar time frame in maintaining the note structure in these two closely related finches. However, the importance of auditory feedback in maintenance of note structure has not been found in any other close-ended learners. Further study is necessary to determine whether the audition-dependent note maintenance is only found in Estrildid finches or, more commonly, in other families of passerines.

### Individual Variation in the Role Played by Auditory Feedback

Individual variation in the rate and degree of the song deterioration observed in this study was remarkably large. Among the six bilaterally deafened

subjects, one showed very little immediate change in song structure, while the other five exhibited various degrees of deterioration in song syntax and the number of note types lost. The resilience of song structure in the one relatively unaffected subject cannot be explained by its age; we suggest that individuals within a species may rely on auditory feedback to a varying degree in producing their adult songs. Similar individual differences in the pattern of song deterioration were reported in adults of other species deprived of auditory or proprioceptive feedback (Konishi, 1965; Bottjer and Arnold, 1984; Tsutsui and Bottjer, 1989). Intraspecific differences in the neural mechanism underlying the production of crystallized songs may be more common than usually supposed. The auditory memory of note syntax may be converted into proprioceptive and/or motor memory to a different extent in different individuals.

### Neurophysiological Implications

Species differences in the effects of auditory deprivation suggest that neural mechanisms underlying song production in adulthood differ among different species. Species that do not rely on immediate auditory feedback to produce normal song syntax may use a mechanism for indexing those notes included in a song that relies on proprioceptive feedback or on an internalized motor memory. In contrast, species that require audition for normal syntax production may rely on dynamic ongoing interactions between auditory feedback and the motor pathway to index an appropriate series of notes. The site of this auditory/motor interaction within the central nervous system of the adult Bengalese finch is unknown, and its identification might reveal the basis for species and individual differences in how auditory feedback interacts with motor pathways.

Among brain nuclei involved in song development and production, bilateral lesion of a thalamic nucleus, nucleus uvaformis, in adult zebra finches results in production of songs with erratic note syntax (Vicario and Williams, 1993), a symptom somewhat similar to that of deafened adult Bengalese finches. It is possible that note indexing is carried out in Uva, although no anatomic connection between Uva and the auditory system has been demonstrated to date. It may be that Uva receives auditory input in Bengalese finches and functions to index notes by interacting with the immediate auditory feedback. Alternatively, Nif, a nucleus that receives both auditory and motor input directly from

field L (the avian equivalent of mammalian primary auditory cortex) and Uva, may be the site of auditory-motor coordination in the generation of song syntax in Bengalese finches.

Another possibility is that nuclei that play crucial roles in the song-learning process that converts auditory experience into motor patterns may continue to be an important part of song production pathway in adulthood in Bengalese finches. For example, LMAN and area X, nuclei in the anterior forebrain song system that are necessary for song learning but not for song production in zebra finches (Bottjer et al., 1984; Sohabji et al., 1990), may continue to be active in generating the vocal motor pattern in adult Bengalese finches. Interestingly, bilateral lesion of LMAN in the adult male canary resulted in a marked loss of note number included in a song soon after the surgery, which implies the involvement of LMAN in adult song production (Nottebohm et al., 1990).

Songs of all close-ended learners have been considered to be generated by a motor tape that does not require moment-to-moment auditory feedback. We showed here that not all close-ended learners can produce songs without real-time auditory feedback; Bengalese finches require moment-to-moment auditory feedback to produce normal song syntax. This audition-dependent song production system may be explained as a function of syntax plasticity maintained by the adults of this species. The manner in which songs are produced in different species of songbirds may be as varied as the variation in their songs. Studies of song variation and species differences in reliance on auditory cues provide powerful tools with which to test hypotheses about the neural mechanisms of songs generated in a few focal species (Kroodsma and Brenowitz, 1996).

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