The Journal of Neuroscience, September 1991, 11(9): 2896–2913

A Comparative Study of the Behavioral Deficits following Lesions of Various Parts of the Zebra Finch Song System: Implications for Vocal Learning

Constance Scharff and Fernando Nottebohm

The Rockefeller University, New York, New York 10021

Song production in song birds is controlled by an efferent pathway. Appended to this pathway is a "recursive loop" that is necessary for song acquisition but not for the production of learned song. Since zebra finches learn their song by imitating external models, we speculated that the importance of the recursive loop for learning might derive from its processing of auditory feedback during song acquisition. This hypothesis was tested by comparing the effects on song in birds deafened early in life and birds with early lesions in either of two nuclei—Area X and the lateral magnocellular nucleus of the anterior neostriatum (LMAN). These nuclei are part of the recursive loop. The three treatments affected song development differently, as reflected by various parameters of the adult song of these birds. Whereas LMAN lesions resulted in songs with monotonous repetitions of a single note complex, songs of Area X–lesioned birds consisted of rambling series of unusually long and variable notes. Furthermore, whereas song of LMAN lesioned birds stabilized early, song stability as seen in intact birds was never achieved in Area X–lesioned birds. Early deafness also resulted in poorly structured and unstable song. We conclude that Area X and LMAN contribute differently to song acquisition: the song variability that is typical of vocal development persists following early deafness or lesions of Area X but ends abruptly following removal of LMAN. Apparently, LMAN plays a crucial role in fostering the kinds of circuit plasticity necessary for learning.

The song control system of songbirds consists of a number of nuclei that constitute the efferent path for the production of learned song (Nottebohm et al., 1976). This efferent path includes the high vocal center (HVC) of the neostriatum, which projects to the robust nucleus of the archistriatum (RA); RA sends a direct projection to the hypoglossal motoneurons (nXIIts) that innervate the muscles of the trachea and the vocal organ (syrinx) (Nottebohm et al., 1976). In addition, there is a circuit, called the recursive loop (Okuhata and Saito, 1987; Williams, 1989), that is necessary for song learning but not for the production of learned song (Bottjer et al., 1984; Scharff and Nottebohm, 1989; Sohrabji et al., 1990). This circuit starts in HVC; HVC sends efferents to Area X of lobus parolfactorius; Area X projects to the medial nucleus of the dorsolateral thalamus (DLM), whose efferents connect to the lateral magnocellular nucleus of the anterior neostriatum (LMAN); LMAN, in turn, projects to RA. Since HVC sends a direct projection to RA, the recursive loop forms an alternate, indirect pathway from HVC to RA. Auditory information reaches HVC via Field L (Kelley and Nottebohm, 1979; Katz and Gurney, 1981). The relation between these various nuclei and pathways is shown in Figure 1.

It is important to understand what each nucleus in the recursive loop contributes to song learning. The present report compares the effects of bilateral lesions of Area X in adult and juvenile zebra finches with the effect of bilateral lesions of LMAN in juveniles and with juvenile deafening. If the recursive loop enables the motor pathways to match vocal output with an acquired model, interruption of the recursive loop at any point—or deafening—might have the same effect. Our results show that this is not the case.

Male zebra finches acquire their song during the first 80 d after hatching (Immelmann, 1969; Eales, 1985). Normally, they imitate components from one or more songs from several they hear during development (Immelmann, 1969; Böhner, 1983; Clayton, 1987; Williams, 1990). Songlike vocalizations are first produced approximately between 28 and 35 d after hatching (Immelmann, 1969; Arnold, 1975). This initial, poorly structured rendering is called subsong. With time and practice, sounds become more stereotyped and are produced in a more or less fixed sequence that matches the tutor model (or models). This final stage in song acquisition, leading to stable adult song, has been referred to as "crystallization." Thereafter, song changes little if at all, even over periods of months or years (Immelmann, 1969).

Deafening (Price, 1979) or lesions of LMAN (Bottjer et al., 1984) or of Area X (Scharff and Nottebohm, 1989; Sohrabji et al., 1990) after crystallization have little effect on retention of the learned pattern of song. In this report, we employ a combination of quantitative and qualitative analysis of different parameters of song to evaluate the nature of the deficits that follow these three types of lesion in juvenile zebra finches.

Materials and Methods

Subjects. Sixty male zebra finches (*Taeniopygia guttata*) were used in this study. All subjects were hatched in our own breeding facilities at the Rockefeller University Field Research Center. Adult and juvenile

---

Received Nov. 19, 1990; revised Apr. 22, 1991; accepted Apr. 25, 1991.

We thank Kathleen Gould and Uta von Rad for technical assistance, Jeffrey Cynx for scoring of sonograms and helpful comments, Stephen Clark for statistical advice, Heather Williams for critical reading, and Marta Nottebohm for editorial help. We also acknowledge the constructive comments of two anonymous reviewers. This work was supported by NIH Grant DC 00182. It was also supported in part by Biomedical Research Support Grant S07RR07065 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

Correspondence should be addressed to Constance Scharff, The Rockefeller University, 1230 York Ave. #137, New York, NY 10021.

males were raised in an enclosed indoor aviary (4 × 2.5 × 2 m). This aviary housed 12 breeding pairs and their offspring and was kept on a 12-hr light/12-hr dark photoperiod. All operated juveniles were returned to the aviary after surgery; they were removed from the aviary after 90 d of age. In some cases, breeding pairs were housed in individual small flight cages (46 × 26 × 24 cm).

Surgery. Surgery was performed under anesthesia induced by methoxyflurane (Metofane, Pitman-Moore), followed by injections of 0.01 ml each of ketamine (Ketalar, Parke–Davis) and xylazine (Rompun, Haver). Injections and lesions were placed stereotaxically according to modified coordinates from the canary atlas (Stokes et al., 1974).

Lesions. All electrolytic (anodal) lesions were bilateral. We used size 000 insect pins (Carolina Biologicals) insulated with Insl-x (Insl-X Product Corp.) as electrodes. For Area X lesions in juveniles, the electrode was angled at 20° pointing rostrally, to avoid passing through nucleus LMAN. Control lesions were outside, but in the vicinity of Area X. For Area X lesions in juveniles, a single penetration on each side was sufficient (90 μA for 90 sec). Area X lesions in adults and LMAN lesions in juveniles were made using three penetrations (Area X, 90 μA/90 sec; LMAN, 40 μA/20 sec). The number of animals by age and treatment type for these and the following experiments are shown in Table 1. Brains were perfused under deep anesthesia with PBS followed by freshly prepared 4% paraformaldehyde. Brains were removed and stored in paraformaldehyde, and 50 μm sections were cut on a vibratome (Lancer) 5–7 d after perfusion. Four brains were embedded in polyethylene glycol and cut on a rotary microtome (Spencer) into 10 μm sections. All sections were stained with a 0.1% solution of cresyl violet acetate (Sigma).

Areas of song nuclei (both Area X and LMAN) were measured on a computer-interfaced microscope (Alvarez-Buylla and Vicario, 1988) in all 50 μm sections that contained a given nucleus. Each cross-sectional area was measured in duplicate (Area X) or triplicate (LMAN). The means of those measures were used to calculate volumes of each side by multiplying the sum of areas by the thickness of the sections. Left and right volumes were averaged. The effectiveness of lesions targeted at Area X and LMAN was expressed in terms of amount of tissue remaining after the operation, expressed as a percentage of the mean volume of Area X or LMAN in eight intact adult animals; for example, if 10% of tissue survived the operation in a recognizable manner the nucleus was considered 90% lesioned.

Volumes of HVC were measured in the same way (to address possible shrinkage of HVC due to retrograde degeneration of cells that project to Area X after lesion).

Deafening. Juveniles were deafened by bilateral removal of the cochlea (Konishi, 1964).

Tracheosyringeal nerve section. The tracheosyringeal branch of the XII cranial nerve (nXIIts) was bilaterally cut (under Metofane anesthesia) at a point midway between the syrinx and the larynx, and 2 mm of nerve were removed.

Fluorogold injections. To check whether lesions targeted at Area X had disrupted the projection from LMAN to RA, 13 birds that had received Area X lesions during development also received bilateral injections of the retrograde tracer Fluorogold (Fluorochrome, Inc.; total volume, 40 nl; 2% concentration) into RA 5 d prior to death. LMAN sections were examined under UV illumination for the presence of retrogradely labeled neurons. This procedure was also employed to judge lesion placement and size in three of the four birds with LMAN lesions.

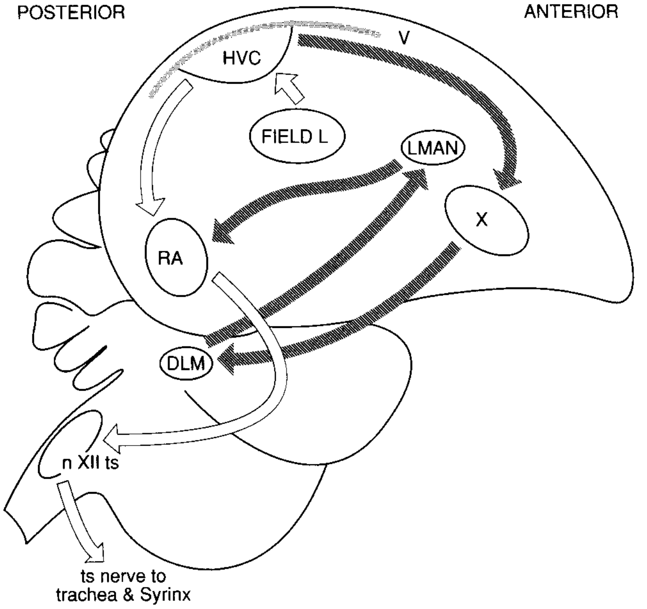

Figure 1. Schematic sketch of sagittal section through adult songbird brain showing the major pathways involved in the production and acquisition of learned song. Nuclei of the descending efferent pathway are connected with *white arrows,* those of the recursive loop with *gray arrows.* Auditory input reaches *HVC* via *FIELD L.* [HVC was originally called the hyperstriatum ventralis, pars caudalis (HVc), a misnomer (Nottebohm, 1987).] See introductory remarks for other abbreviations; *V,* lateral ventricle.

Description and terminology of adult song. Male zebra finches sing when courting a female (directed song). The same song is also produced by males kept by themselves (undirected song) (Hall, 1962; Immelmann, 1968). The patterns used in both situations are very similar (Sossinka and Böhner, 1980).

Adult song begins with several renderings of a same introductory note, followed by a set of dissimilar notes. The latter notes are rendered in a stereotyped sequential order and constitute the "motif." A motif lasts approximately 700 msec (Sossinka and Böhner, 1980), with frequencies ranging from 0.5 to 8 kHz (Fig. 2*C*).

A song note is defined as a continuous, morphologically discrete trace on a sound spectrogram. The introductory notes that initiate song are very similar among males; in any one male their morphology is very stereotyped, though their number and temporal spacing vary (Sossinka and Böhner, 1980). The number of motif notes (5–11 in this study) and their structure and duration (9–182 msec in this study) differ between individuals, resulting in motifs that are specific for each bird. Song is produced in strophes, that is, introductory notes followed by a variable number of repetitions of a same motif. Strophes are separated by silent intervals, usually 0.5 sec or more.

The durations of successive renderings of a same song note and of the intervals between particular song notes vary on the average by less than 5 msec. Some males have two motifs, one being an expanded version of the other.

In addition to song, zebra finches also use a variety of calls, for example, the "distance" and the "short" call (Price, 1979; Zann, 1985;

Table 1. Numbers and ages of subjects used for each treatment group

Treatment group	Experimental	Control	Age (range) at operation (d)	Age (range) at song analysis (d)
Early juvenile Area X lesion	17	4	37 (31–46)	128 (95–155)
Late juvenile Area X lesion	6		64 (61–70)	135 (108–157)
Adult Area X lesion	8	8	Older than 100	22 (2–54) d later
LMAN lesion	4	1	43 (40–47)	94 (82–112)
Deaf	4		28 (26–29)	331 (186–383)
Intact		8		119 (92–149)
nXIIts section/intact		5	125 (94–150)	127 (95–153)
nXIIts section/early juvenile Area X lesion	9		130 (95–157)	132 (96–159)

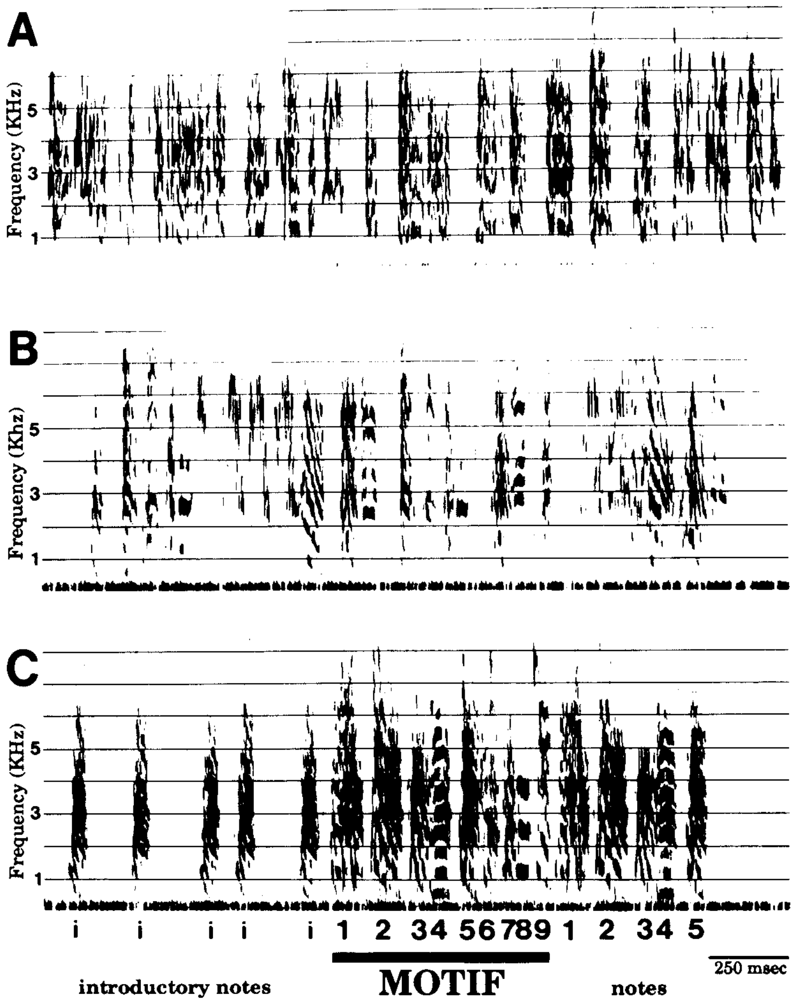

Figure 2. Song development in an individual male zebra finch (lb/y15) showing representative examples of subsong recorded at 36 d (*A*), plastic song at 42 d (*B*), and adult, stable song at 94 d (*C*) (see *Stages in song development* for details).

Simpson and Vicario, 1990). Only the male "distance call"—which birds use when they are visually isolated—is affected by learning and was for this reason included in the sound analysis.

Vocalizations of birds that had been deafened or had brain lesions were considered songlike if they contained repeated motifs and could be elicited by presenting the male with a female.

Stages in song development. The stages of song development of zebra finches have been previously described (for review, see Slater et al., 1988). For the purpose of this study, "subsong" refers to the earliest song productions, consisting of quiet bursts of sound given with variable structure at irregular intervals (Fig. 2*A*). "Early plastic song" describes song that contains some notes of adult appearance while others remain more amorphous. At this stage, there is a tendency for some notes to appear in stereotyped sequential order, although a high variability in both sequence and structure still remains (Fig. 2*B*). During "late plastic song," a stereotyped motif has been developed and the majority of notes have near adultlike morphology. The main changes from this stage to adult "stable" song (Fig. 2*C*) are a crisper definition of notes (i.e., intervals between notes will become sharply defined and completely silent), a shortening of notes and intervals, a downshift in the fundamental frequency of some notes, and an increase in sequence stereotypy.

Sound analysis. Song and calls were recorded on Marantz PMD 221 tape recorders at 4.75 cm/sec through a cardioid dynamic microphone (Realistic 33-992C) placed at a constant distance from the perch of the recording cage. Recording levels were held constant for all birds in order to provide a rough estimate of song loudness. To reduce background noise and control visual contact with other birds, subjects were placed in a double-walled, sound-insulated, transparent Lucite box during recording sessions. Birds that had received Area X lesions in adulthood were recorded three times before lesion and then weekly postlesion, for up to 8 weeks. Recordings of nerve-sectioned animals were made no later than 3 d after surgery. This ensured that nerves had not regrown. Eight juveniles were recorded prior to Area X lesions, three prior to LMAN lesion. After surgery, birds were recorded periodically during development. Song was mainly recorded during undirected singing episodes, but all experimental animals were also presented with a female and directed song was recorded and/or observed.

For detailed analysis of song of birds that received lesions of Area X or LMAN or were deafened as juveniles, only undirected songs of birds that were older than 90 d were used. The following parameters were analyzed: (1) stereotypy of note order within song motifs, (2) morphology of notes, (3) duration of (a) notes and (b) intervals, and (4) variability in the duration of notes and intervals from rendition to rendition. To study variability, both standard deviation (SD) and coefficient of variance (COV) from the mean note and interval length were calculated. The standard deviation, rather than the coefficient of variance, was chosen as the measure for variability because we established in the intact population that the variance was not linearly related to note or interval length (notes, $R^2 = 0.0017$, $p = 0.7463$; intervals, $R^2 = 0.057$, $p = 0.1289$). While the range of note and interval length in early Area X–lesioned animals was much greater than in intact birds, this did not bring about a significant relation ($p < 0.05$) between SD and note length in 10 out of 13 animals (for intervals, 11 out of 13). However, all statistical tests involving variability measures were performed on both SD and COV data. Where significant differences concerning SD are mentioned in the text, they were also found for COV.

The average number of measures performed per note or interval was 17 (total of 9355 individual measures).

For purposes of detailed analysis, all songs of one session were first transcribed with a real-time, fast Fourier transform analyzer (10 mm/sec; model 4500, Multigon). Then representative sonograms of the same material were visually analyzed (125 mm/sec; digital sonogram, model

7800, Kay). Finally, we used the MacRecorder® sound system (Farallon®) in combination with a Macintosh SE/30 computer to digitize, Fourier transform, display, and measure sounds as amplitude envelopes, sonograms (gray-scale map of the relative strengths of the frequencies, plotted against time), and spectrograms [graphs of the relative strength of frequencies at one time point, plotting frequency (Hz) against power (dB)].

Identity of each note was ascribed on the basis of sonogram morphology, spectrogram features, and place within the song. In normal adult zebra finch song, these parameters can be used to identify notes in an unambiguous manner. Since some level of stereotypy is preserved even after juvenile Area X and LMAN lesions and after juvenile deafening, it was relatively easy to assign a note identity to most of the song sounds recorded. In ambiguous cases, notes with similar morphologies on sonograms could often be distinguished by a characteristic shape of their amplitude envelopes. In cases where combined analysis of sonograms and amplitude envelopes was not sufficient to identify a note, notes were also analyzed spectrographically. Very few notes could not be clearly identified when combining these three levels of analysis. In the animal with the most severely disturbed song (where note sequence was the least stereotyped and notes resembled each other in morphology), only 12 notes of 731 analyzed could not be ascribed an identity.

After notes were identified, lengths of notes and intervals were measured from amplitude envelopes with the SoundEdit program of the MacRecorder software. Notes and intervals were measured consecutively; for example, the end of each note would be the beginning of its subsequent interval.

The mean, standard deviation, and coefficient of variance were calculated for each note and interval. Standard deviation was corrected for bias of sample size using Gurland and Tripathi's (1971) correction factor. The measuring resolution of the MacRecorder program at the magnification used was 0.5 msec.

Based on normal zebra finch song characteristics, we quantified song stereotypy in two ways: *sequence linearity* and *sequence consistency.* These two measures address related but different aspects of sequence stereotypy.

Sequence linearity addresses the way notes are ordered in a song. The "sequence linearity score" is expressed as

$$S_{\text{linearity}} = \frac{\text{\# different notes/song}}{\text{\# transition types/song}}.$$

In a completely linear song sequence, each note has only one transition type, that is, is followed by only one other note (or end), and thus this ratio would equal 1. In the case of typical intact zebra finch song, the score tends to be close to but not equal to 1. This is because some notes usually have two transition types, resulting in less linear song with scores smaller than 1 (e.g., an introductory note can be followed either by another introductory note or by note 1, and frequently the last note in a song either terminates the song or goes on to the first note).

Sequence consistency does not address how the notes are ordered but how often a particular path is actually followed. The "sequence consistency score" expresses the frequency with which a main, or typical, sequence appears. The typical transition type for each note is operationally defined as the one most frequently encountered (for introductory notes, the two most frequently encountered). Sequence consistency is thus expressed as

$$S_{\text{consistency}} = \frac{\Sigma \text{ typical transitions/song}}{\Sigma \text{ total transitions/song}}.$$

Complete consistency is thus represented by a score of 1; songs that are less consistent have scores smaller than 1.

A *stereotypy score* was calculated as

$$S_{\text{stereotypy}} = \frac{S_{\text{linearity}} + S_{\text{consistency}}}{2}.$$

Complete stereotypy yields a value of 1; increasing absence of stereotypy approaches 0.

To derive linearity and consistency scores, several songs from one recording session of each individual bird were analyzed (average and range of strophes transcribed per bird: intact, 12, 9–16; juvenile Area X lesion, 20, 7–39). The linearity score for each bird reflects the combined number of different notes and their associated transition types for the several songs analyzed. To calculate the consistency score for each individual, the total number of times that each transition type occurred across the several songs analyzed was used to calculate the proportion of the most common transitions (average and range of each transition type compiled per bird: intact, 15, 11–17; juvenile Area X lesion, 41, 14–86).

To address less quantifiable song features—for example, note morphology—and to assess if song from animals that had received Area X lesions appeared "abnormal" to observers that were familiar with intact zebra finch song, we designed blind tests: two judges were asked to sort sonograms into before/after (for adult lesions) or lesion/intact (for juvenile lesions) categories. Interobserver reliability on the two blind tests was 70 and 77%, respectively.

Intraobserver reliability for measures of note and interval length was checked by repeating the length measurements for one bird. Mean note length between measurements differed by less than 2%.

Results

Histology

Sixteen birds had lesions targeted at Area X in adulthood. In eight of these birds, the lesions affected tissue mainly outside of the target area (mean and range of Area X destroyed: 17%, 7–30%) and were treated as controls. The lesion sizes in the other eight adult birds in this group were in the range (mean and range: 65%, 44–92%) that produced song abnormalities in juvenile Area X lesions (see below). In three of the latter eight birds, LMAN had also been affected by the lesion. Those birds were not discarded from the analysis since LMAN is not essential for song production in adult zebra finches (Bottjer et al., 1984).

Seventeen juvenile birds received lesions of Area X; of these all but four had their LMAN intact (Fig. 3). The four birds in which the lesion encroached on LMAN were excluded from further analysis, since a compromised function of LMAN could contribute to song abnormalities (Bottjer et al., 1984). The smallest early juvenile Area X lesion that produced abnormal song involved only 30% of Area X's volume. Song abnormalities were most pronounced in animals in which more than 80% of Area X was destroyed ($n = 7$). Lesions that affected 30–80% of Area X produced a range of song deficiencies ($n = 6$; Table 2).

In three of the four LMAN-lesioned birds, the lesion destroyed all of LMAN; in the fourth bird 65% of LMAN was affected. One control lesion was comparable in size to the others but caudal to LMAN.

Song description of subjects with Area X lesions

Adults

Song production and quality appeared to be unaltered after lesions of Area X in adults up to 54 d after surgery (mean and range: 22 d, 2–54; Fig. 4). This was true even when the lesion targeted on Area X also encroached on LMAN (cf. Bottjer et al., 1984). Control birds that had lesions of comparable size but mainly outside of Area X also did not show any changes in song.

Two judges that were blind to the treatment and were asked to sort representative sonograms into before/after lesion categories did not perform better than chance (48% and 50% correct answers, respectively) and found the songs very similar. Therefore, these songs were not subjected to further analysis (i.e., as done for juvenile lesions; see below). Thus, we cannot rule out that adult Area X lesions might subtly affect note or interval length or the variability of these parameters. But if they did, it would be on a much smaller scale than in lesions in juveniles, where those changes were readily apparent to the eye. The sonograms of other vocalizations (distance and short calls) also looked normal.

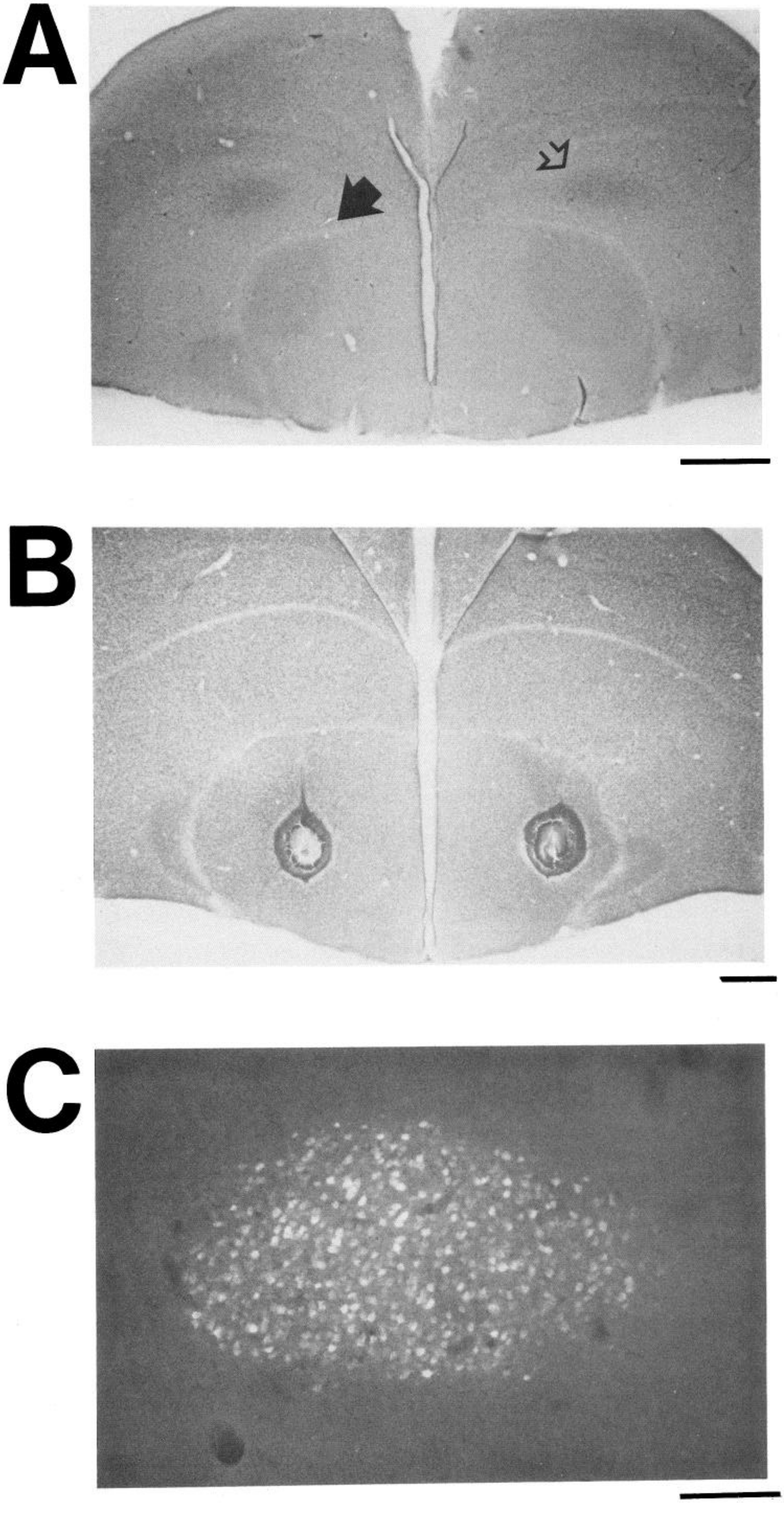

Figure 3. Photomicrographs of Area X, LMAN and lesion site. Frontal sections (50 μm in thickness). *A*, The darker staining, pear-shaped Area X (*solid arrow*) and the oblong LMAN (*open arrow*) are prominently visible in cresyl violet–stained tissue. *B*, Section of one of the juvenile males that received a lesion targeted at Area X as juvenile (lb/y14). Some tissue spared by the lesion is visible dorsal to the lesion sites. *C*, LMAN was not affected by the lesion: right LMAN of same animal as in *B* backfilled from RA with retrograde tracer Fluorogold, photographed under UV illumination. Section is at a more rostral plane than *B*. Scale bars: *A* and *B*, 1 mm; *C*, 200 μm.

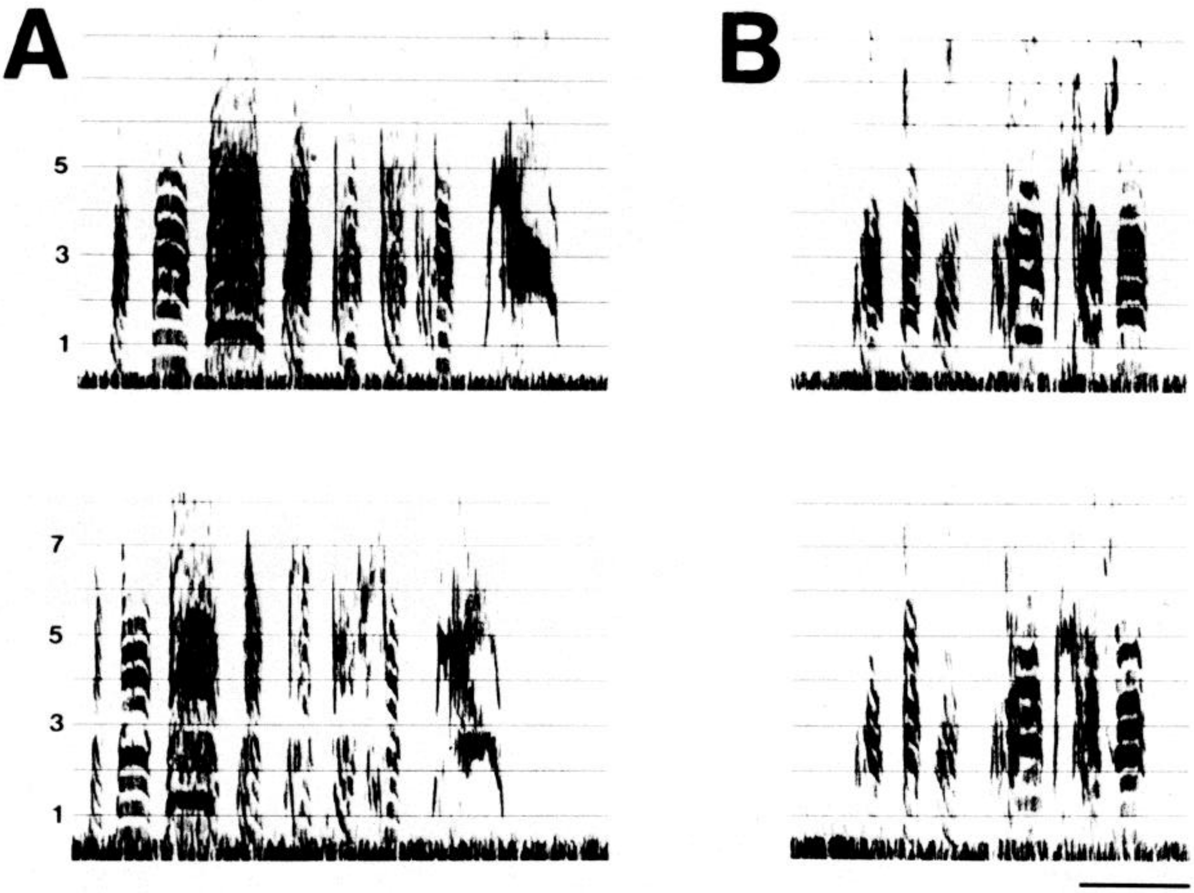

Figure 4. Sonograms of two birds, lb/db7 (*A*) and lb/or30 (*B*), that received Area X lesions in adulthood exemplify that songs before the lesion (*top two panels*) did not appear different from songs after lesioning (*bottom*) either 16 d (*A*) after the operation or 45 d after (*B*). *A*, Song shown in the *bottom panel* was delivered at a faster speed than the song in the *top panel*, because the male was directing that song toward a female. This is a typical difference between directed and undirected song and not a product of the lesion. All other panels show undirected song. *Vertical scale* at left shows frequency in kHz. Scale bar, 250 msec.

Table 2. List of birds that received Area X lesions as juveniles

				Song					
Bird band	% Lesioned	Location of lesion	Age (d)	Note		Interval		Morphology (abnormal notes/ total notes)	Distance call
				Duration	Variability	Duration	Variability		
red49	>80	Ventral/central	37	+	+	+	+	4/4	*
red46	>80	Ventral/central	39	+	+	NS	+	4/5	*
red45	>80	Ventral/central	40	+	+	+	+	4/5	*
red42	>80	Ventral/central	40	+	NS	+	NS	4/5	*
lb/y14	85	Ventral/central	46	+	+	+	+	6/10	*
blk25	85	Ventral/central	31	+	+	+	+	10/11	*
dg10	84	Central	31	+	+	+	+	12/13	*
or98	78	R:medial L:ve.lat.	36	NS	+	+	+	2/5	nc
lb/r95	62	Ventromedial	38	NS	+	NS	+	2/9	*
red94	49	R:tiny L:cent.lat.	35	−	NS	NS	NS	0/10nc	nc
lb/r78	43	Ventral/central	36	+	+	+	+	5/6	nc
wh/blk34	32	Central	38	NS	NS	NS	NS	0/13 nc	nc
red19	30	Ventral	32	NS	+	+	+	6/9	*

The % lesioned column refers to the mean of right and left Area X affected. The brains of the first four birds were sectioned at 10 μm, which in comparison with material sectioned at 50 μm made it harder to visualize Area X's boundaries. Since with this method the area estimated to be intact was in the 0–10% range, at least 80% of Area X of those birds was lesioned. Song impairment was, on average, greatest in birds with lesions of >80%. In the note and interval columns, a plus (minus) sign indicates a significantly higher (lower) value than the intact group; NS indicates no significant difference (see Materials and Methods for details). In the morphology and distance call columns, nc indicates normal morphology, and an asterisk indicates abnormal morphology. Number of notes includes the introductory note.

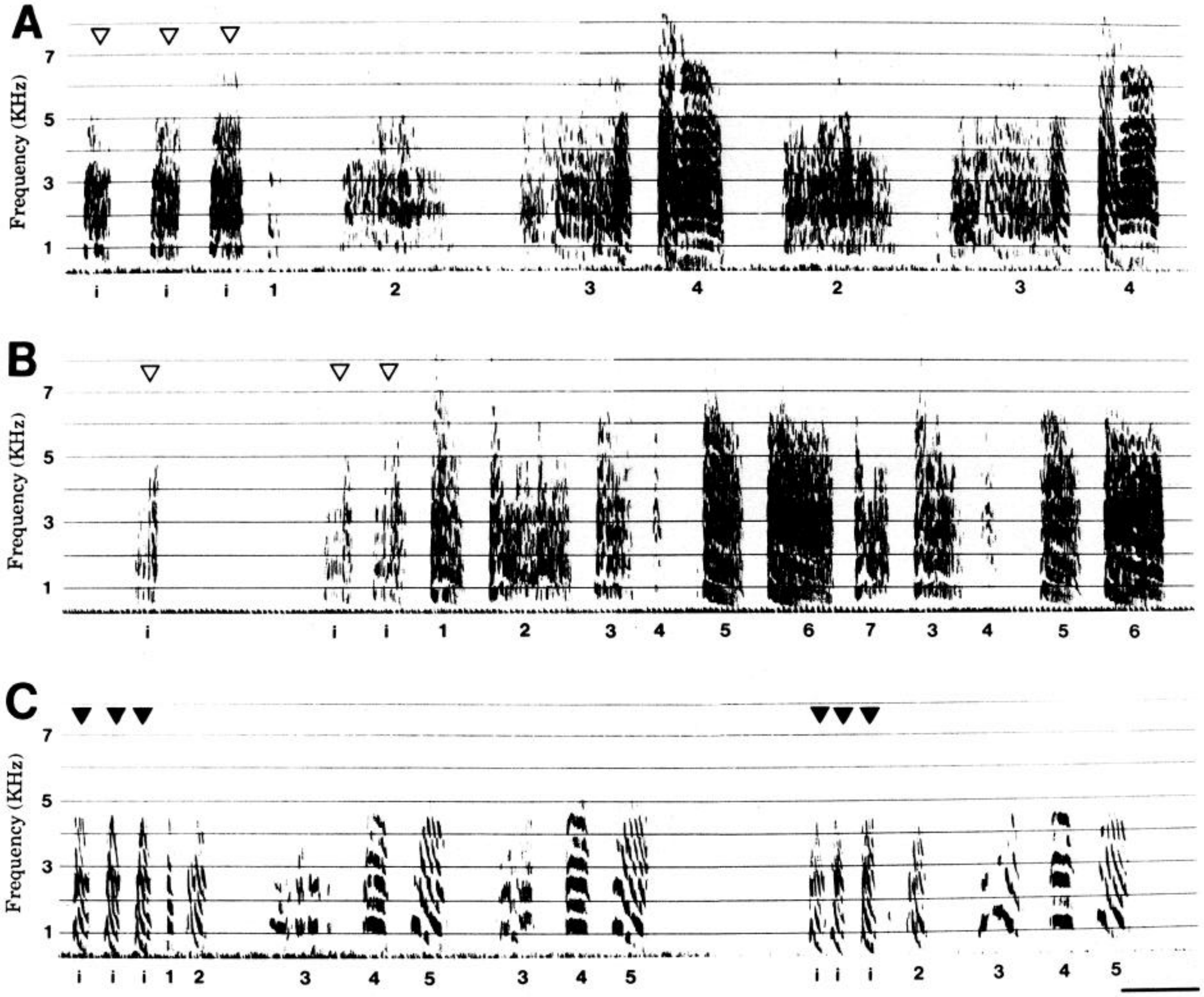

Figure 5. Sonograms of three individuals—lb/r78 (*A*), dg10 (*B*), and or98 (*C*)—that sustained Area X damage of 43%, 84%, and 78%, respectively, illustrate typical abnormalities seen after Area X lesions. In *A* and *B*, note and interval length and the variability of those measures were different from those of intact song; in addition, notes were of abnormal morphology but retained recognizable identity (numbers on x-axis) and were strung together with some degree of stereotypy. *C* illustrates song that was less severely affected, i.e., four of five notes had relatively normal morphology and length characteristics. One note (*3*) was very unstable; in addition, interval length and variability of duration of all notes were abnormal. Also note the existence of introductory notes that can be of abnormal (*open triangles*) or normal (*solid triangles*) morphology. Scale bar, 250 msec.

Early juvenile lesions

Juveniles with Area X damage developed severely abnormal song. Figure 5 shows representative sonograms of songs from birds that had sustained damage to Area X during development. As shown in Table 2, 11 out of 13 males that received lesions between 30 and 46 d developed song that exhibited clearly abnormal features. Of the two birds with only minor song abnormalities, one (red94 in Table 2) had a large lesion in its left side (80%) but received only minimal damage to its right Area X (18%). The other bird's (wh/blk34, Table 2) lesion was also small (32%), but bilaterally symmetrical and in the range that produced severe song abnormalities in still another bird (red19, Table 2). Four control birds with lesions that mainly or exclusively affected regions outside of Area X did not show song abnormalities. These lesions were comparable in size to others that were on target and caused song abnormalities.

The results from the blind observer test confirm the severity of the song abnormalities. Both judges recognized song as belonging to birds with lesions or intacts with more than 80% accuracy.

We will now describe in greater detail the effect on song of lesioning Area X in juveniles.

Duration of notes and intervals and variability of duration. The distribution of note and interval lengths (Fig. 6) and measures of variability (Fig. 7) differed between intact birds and birds that had received Area X lesions as juveniles. In all cases, Area X lesions brought about a significant increase in the mean value of these parameters (two-tailed unpaired *t* test, $p = 0.001$).

Individual birds with early Area X lesions tended to have longer and more variable elements (i.e., notes and intervals) than those of intact birds. In addition, the same individual could also have elements of similar length and/or length variability as those found in the intact population (e.g., dg10 in Figs. 6*C*, 7*C*). However, the higher variability of notes and intervals was not partial to unusually long elements: when the variability of elements from the intact population was compared to the variability associated with only normal-length elements of the Area

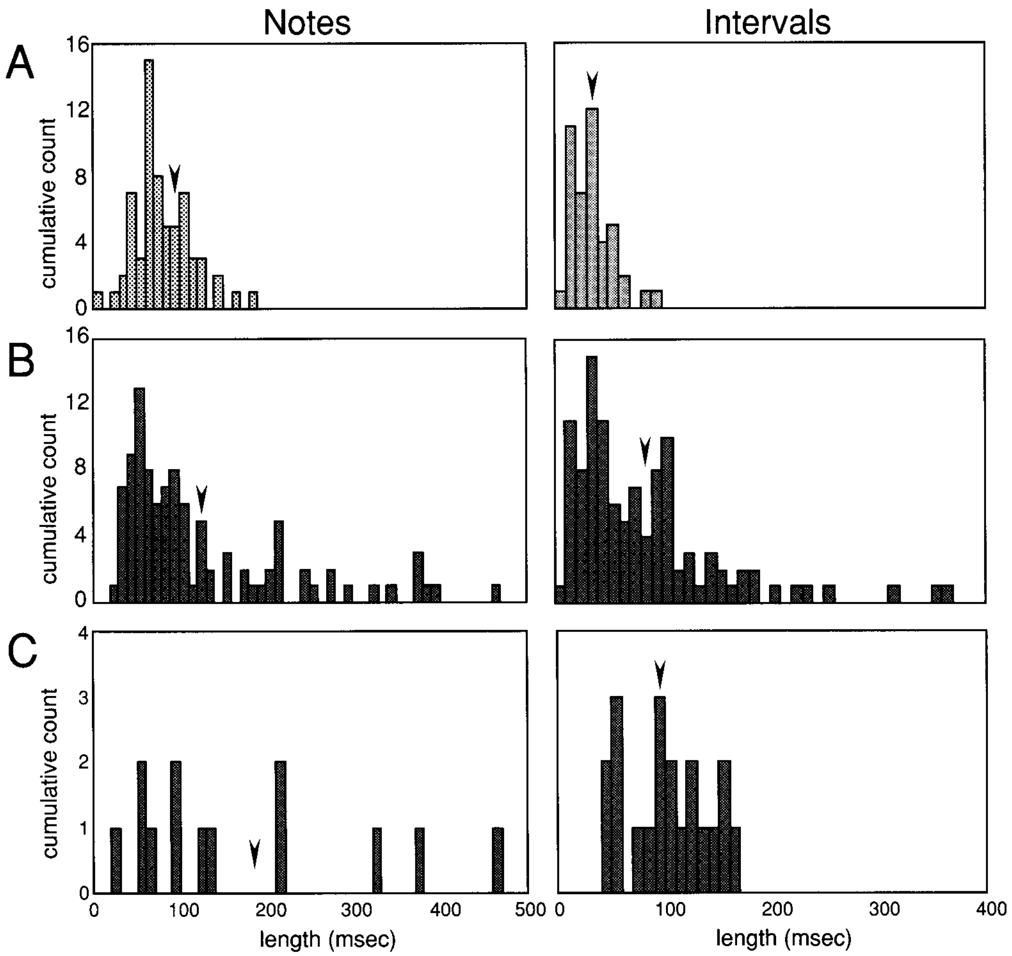

Figure 6. The frequency distributions of note and interval durations of all intact birds (*A*) differed from those of all early Area X-lesioned animals (*B*). In the *left panel* of *A*, the mean lengths of 64 notes of eight intact animals show a frequency distribution with a mean of means (*arrowheads*) at 81 msec. In contrast, the mean lengths of 101 notes from birds that received Area X lesions as juveniles (13 individuals) had a frequency distribution shifted toward longer note lengths (mean of means, 126 msec) as shown in the *left panel* of *B*. The same pattern was seen for interval lengths in the *right panels* of *A* and *B* (intacts: mean of means of 44 intervals, 36 msec; Area X lesions: mean of means of 109 intervals, 83 msec).

Panel C compares one individual (dg10) that received an early Area X lesion to the intact population in *A* and highlights the fact that Area X-lesioned animals tended to have notes and intervals of abnormal duration and variability *in addition* to notes and intervals of normal duration and variability. Notes and intervals in *B* and *C* were significantly different from *A* at $p = 0.001$ (unpaired two-tailed Student's *t* test).

X-lesioned animals, the difference in variability was still significant at $p = 0.0001$.

Analysis of individual birds furthermore revealed that Area X lesions could bring about song that was abnormal in only one or two of the four parameters analyzed. Note duration and variability might fall within the normal length range while those parameters were uncommonly long and variable for intervals (e.g., bird lb/r95 in Table 2).

This latter finding suggests that length of notes and intervals and the stability of these parameters can develop independently from each other.

Morphology of notes. All notes from one representative song of each intact and each lesioned bird were used to construct libraries of the notes of intact ($n = 64$) and Area X-lesioned ($n = 101$) birds. Most notes of intact adult zebra finches fell into one of four morphologically readily classifiable categories (Price, 1979). Examples of these are shown in Figure 8*A*: (1) harmonically related stacks, similar to the short-call, (2) fast frequency modulated downsweeps, as is typical for introductory notes, (3) long-call-like combinations of the previous two types, and (4) high notes. Some notes (5) appear to be a complex mixture of different components.

Of the notes from early Area X-lesioned animals, 44% had equivalent types in the library assembled from intact animals. Representative examples of these are illustrated in Figure 8*B*. All of these normal looking notes fell into categories (1), (2), or (3). The remaining 56% had morphologies that were atypical in that they had very wavering fundamental frequencies that when accompanied by their corresponding harmonics gave a "noisy" appearance, as exemplified in Figure 8*C*. Such characteristics could reflect poor frequency control by individual sound sources and/or the simultaneous activation of multiple sound sources. Sounds of this kind are not typically encountered in intact adult animals but do occur in plastic song (compare Fig. 2*A* with Fig. 8*C*).

Motif stereotypy and strophe length. Early Area X-lesioned animals showed significantly less sequence linearity and sequence consistency than intact birds (Fig. 9*A,B*). Animals with more damage to Area X tended to have less sequence stereotypy than birds in which the lesion had destroyed less of Area X (Fig. 9*C*).

The number of different note types that followed each song note differed between intact adults (mean, 1.4; range, 1.1–1.9) and early Area X-lesioned birds (mean, 2.6; range, 1.5–4.2). This indicates that note order in the lesioned birds was less linear than in intact birds, but clearly not random. Moreover, even in lesioned birds many note combinations appeared with a high degree of consistency, resulting in a high frequency of transitions between particular note pairs. In lesioned birds, these typical transitions constituted on average 75% of all transitions (range, 53–92%), whereas in intact birds this proportion was 98% (range, 93–100%).

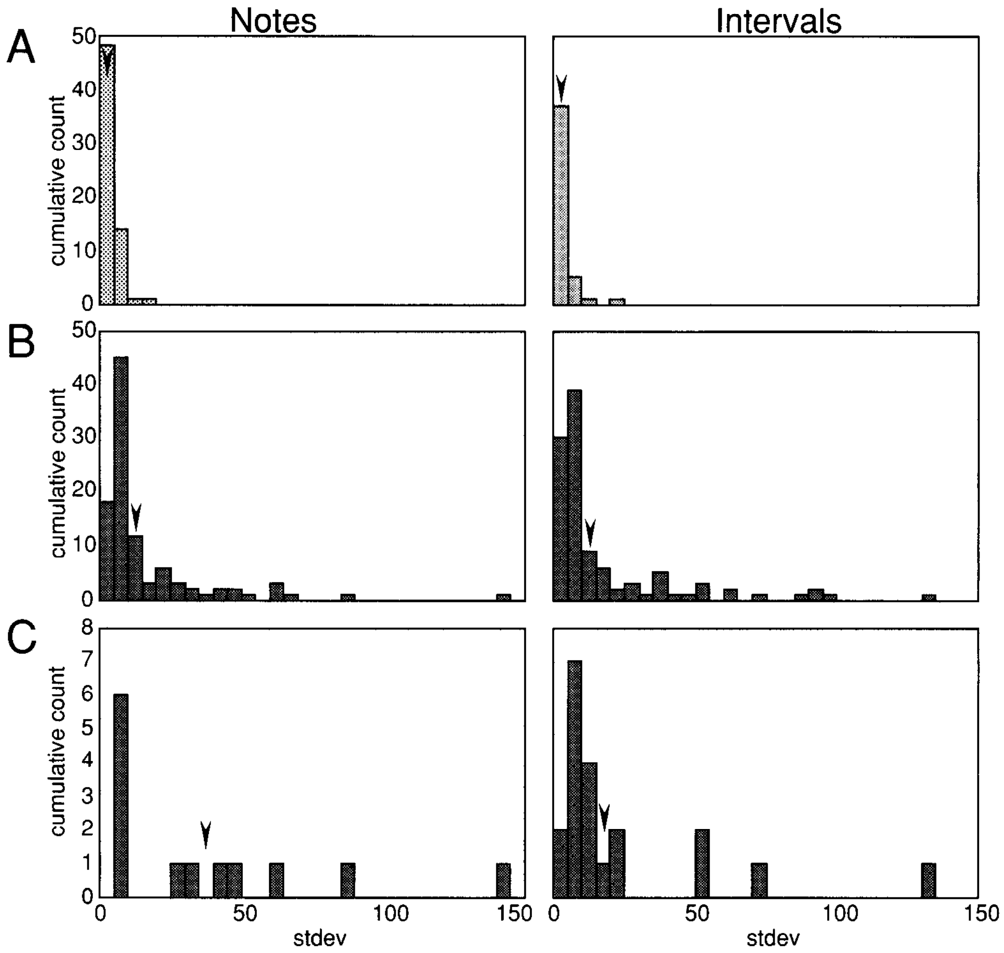

Figure 7. Frequency distributions of variability measures of all intact animals (*A*), all early Area X–lesioned animals (*B*), and one early Area X–lesioned individual, dg10 (*C*) illustrate the same trend for variability of note and interval lengths as shown in Figure 6 for durations. *Arrowheads* indicate mean values. Intact birds' notes and intervals varied very little from rendition to rendition, whereas some notes and intervals of Area X–lesioned animals varied considerably. As shown in the individual bird dg10 that received an early Area X lesion (*C*), some notes and intervals exhibited normal variability while others varied dramatically (mean of SDs of note duration measures: intact animals, 4; Area X–lesioned animals, 16; mean of SDs of interval duration measures: intact animals, 4; Area X–lesioned animals, 19). SDs in *B* and *C* were significantly different from *A* ($p = 0.001$, unpaired Student's *t* test).

Animals with little motif stereotypy sang abnormally long strophes, sometimes (e.g., dg10) lasting as long as 25 sec. The longest strophe measured from the intact birds lasted 7 sec.

Other typical features of the birds with juvenile lesions of Area X. The song of 11 of the 13 early lesioned males began with notes that had characteristics of introductory notes; they were short, repeated, and produced with varying temporal spacing before the motif. In seven of those birds, the morphology of introductory notes was like that of intact zebra finches (Sossinka and Böhner, 1980). In the remaining six, the morphology of these notes was unlike anything seen in intact birds (illustrated in Fig. 5).

The song of the birds that received Area X lesions as juveniles was lower in volume than the song of intact males. In contrast, distance calls were produced at volumes equivalent to those of intact males, as judged by the volume settings on the audio equipment used to produce sonograms of songs from intact animals.

The average number of notes in the lesioned birds' repertoire was not significantly different from that of intact animals (see Fig. 13*C*). The morphology, duration, and variability of duration of distance calls were abnormal only in a subset of those males whose song was abnormal (Table 2, Fig. 10).

Area X lesions during late development

Males that were recorded during late plastic song received Area X lesions between the ages of 60 and 70 d. Table 3 shows the respective lesion size, age at operation, and adult (postlesion) song characteristics of those birds. In two birds, the lesion brought about a clear deviance from the intact adult population in most measured parameters. One of those birds (dg8) with severe song abnormalities had an asymmetric lesion with a mean lesion size of only 25% (left, 19%; right, 81% lesioned), while another bird's song (lg101) was not different from intact adult birds in any respect in spite of complete bilateral destruction of its Area X.

To assess whether the developmental stage of prelesion song played a role in determining the lesions' effect on song, we analyzed prelesion recordings of plastic song when the birds were between 57 and 66 d old. At that time, all birds had individually recognizable notes that varied in their degree of morphological maturity. Figure 11 compares each bird's pre- and postlesion values for various song parameters with the data of the intact and early Area X–lesioned population.

Figure 11*C* illustrates that song of all birds had intervals of normal duration before the lesion. This parameter did not change in any subject after the lesion. Similarly, in birds whose plastic song had already achieved normal adult note duration, the lesion had no significant effect on note length (Fig. 11*A*). However, note duration stayed abnormally long after Area X lesions in those birds whose note lengths in plastic song were significantly longer than in intact adults.

Variability of note and interval duration (Fig. 11*B,D*) during plastic song was significantly higher than in intact adults in all but one bird. After the lesion, this variability decreased in some

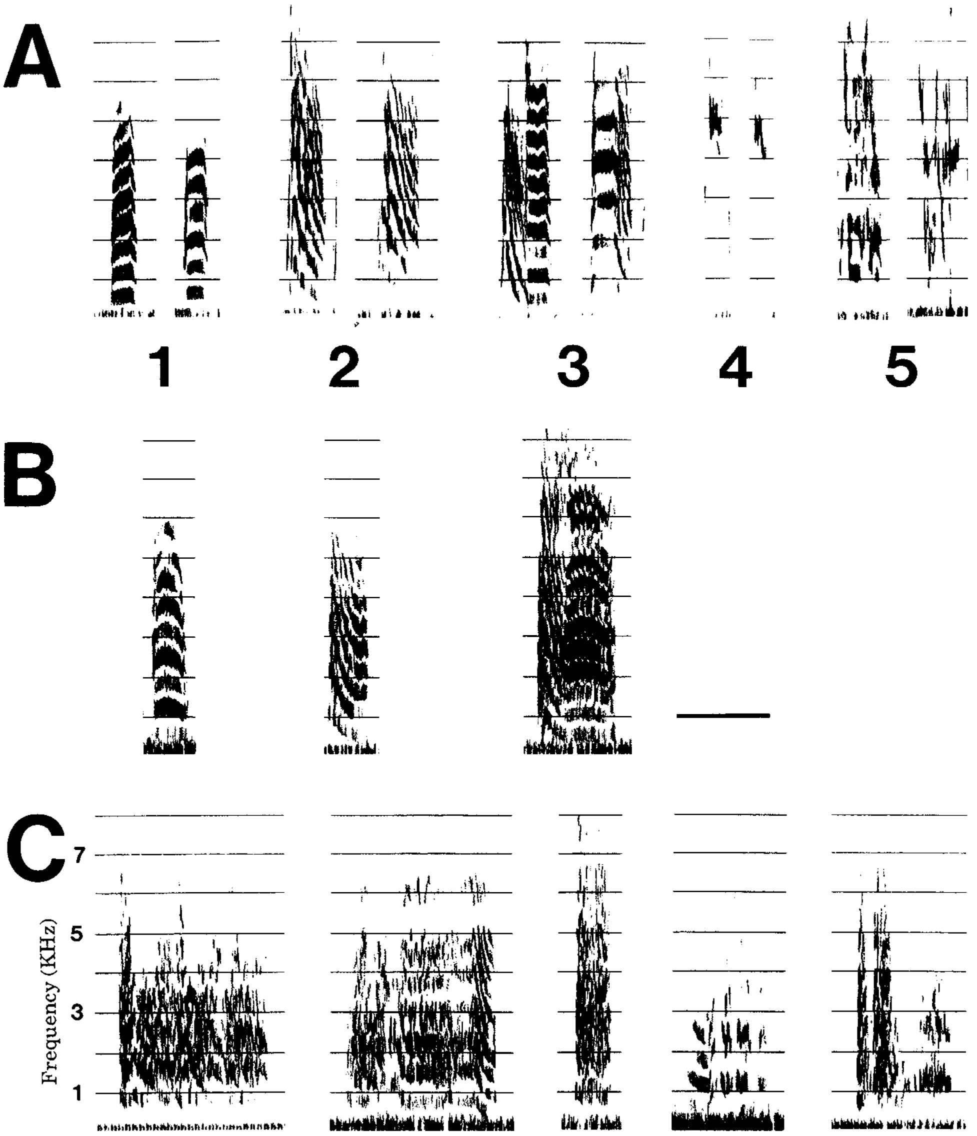

Figure 8. Intact adult zebra finches have characteristic note types (*A*). Of all notes in songs of early Area X–lesioned animals, 56% exhibited morphologies not normally encountered in intact song. Examples of those abnormal notes are shown in *C*. The remainder of the notes were fairly normal and fell in one of the categories shown in *A* (*1–5*; refer to Results). Exemplars of normal notes from Area X–lesioned animals are shown in *B*. Scale bar, 250 msec.

birds to levels typical of normal adult birds but remained significantly different from intacts in others.

A similar pattern was observed for sequence consistency and sequence linearity; while five birds showed consistency scores during plastic song that were outside of the intact adult range, only two of those remained abnormal with respect to this parameter after the lesion.

The total number of notes in the song repertoire did not vary dramatically before and after the lesion (mean change, minus 0.3 notes; range, − 3 to + 1).

In summary, song parameters that were already characteristic of intact adults were least affected by Area X lesion late in development. The developmental stage of note duration may be a good predictor of the extent to which other parameters of song will show normal maturation after a lesion of Area X late in development.

Early LMAN-lesioned and early deafened juveniles

As reported previously, lesions of LMAN or deafening in juvenile male zebra finches (Price, 1979; Bottjer et al., 1984) resulted in severe disturbance of song acquisition (Fig. 12). We wanted to see to what extent song of early Area X–lesioned animals differed from song of early LMAN-lesioned and early deafened birds.

The most striking effect on song after LMAN lesion was a significantly reduced number of different notes (Fig. 13*C*) and the fact that these notes were strung together in highly stereotyped repetitive sequences. In contrast to the songs of early Area X–lesioned birds, the songs produced by birds that received LMAN lesions had normal note and interval duration and normal note variability. However, the duration of intervals was significantly more variable than in intact birds. Judging note morphology by the same criteria employed to judge the appearance of notes from early Area X–lesioned animals, only 4 of 14 notes produced by the LMAN-lesioned animals had no equivalent in the library of notes from intact animals.

One bird in which the lesion affected tissue caudal to LMAN but left LMAN itself intact did not show any abnormalities in song development.

Zebra finches that underwent early deafening produced as adults songs composed of notes that were significantly shorter and less stable than those of intact birds. Intervals between song

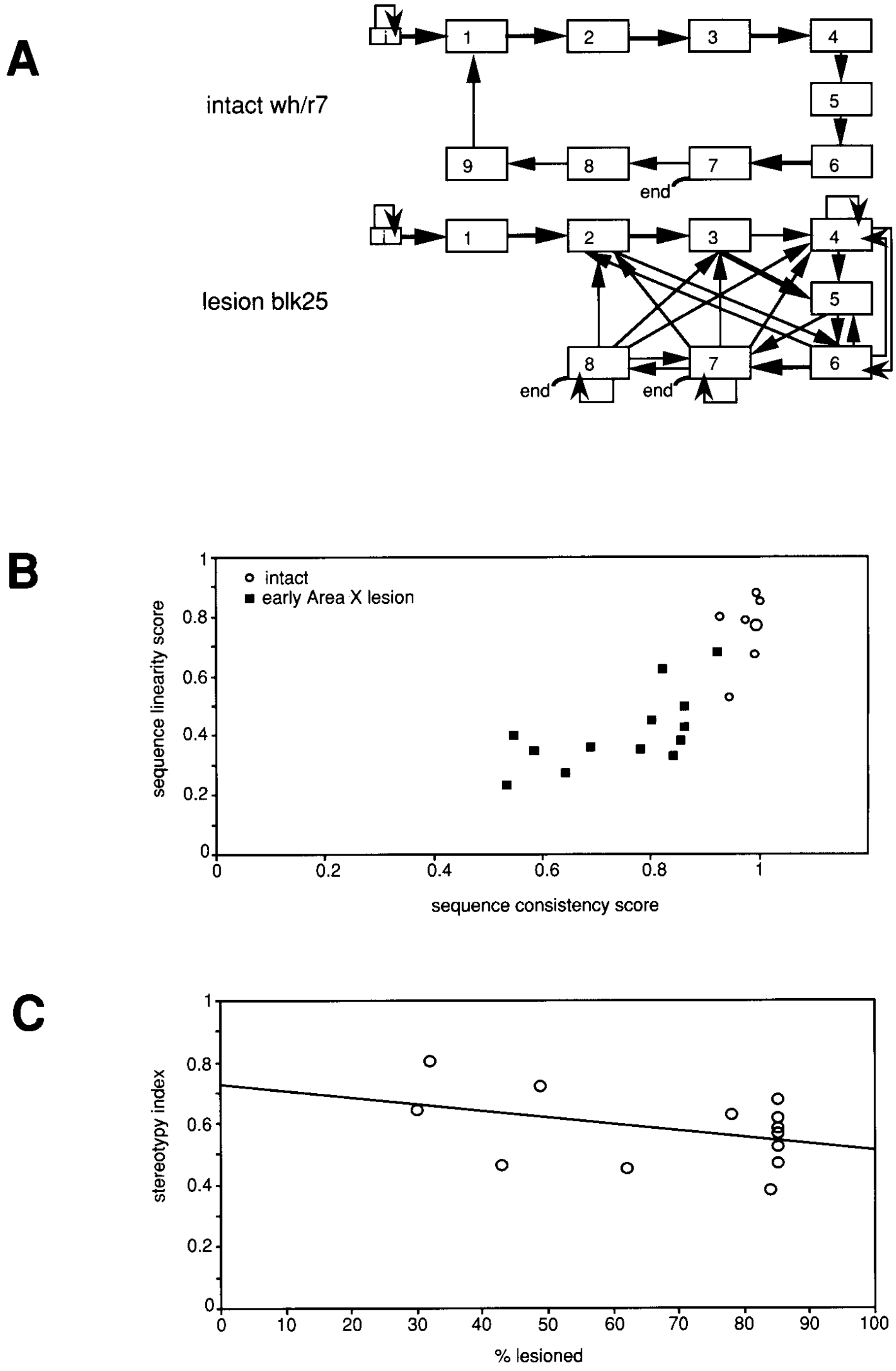

Figure 9. Intact birds had a higher degree of song stereotypy than early Area X–lesioned birds. *A,* Representative flow diagrams of one intact and one lesioned bird. The relative frequencies of transitions between notes, which are represented by *boxed numbers,* varied as indicated by the different widths of the *arrows.* End of strophes are indicated by *small curved lines.* Total notes used to compose sequence diagram: *wh/r7,* 174, *blk25,* 411. *B,* Intact bird's song is linear and consistent (*high scores, open circles*). The less linear and less consistent songs of birds that received early Area X lesions have lower linearity and lower consistency scores, indicating that notes frequently appeared in more than one combination (*filled squares*). (Mann–Whitney U test, $p = 0.001$, $U_{\text{consistency}} = 0$, $U_{\text{linearity}} = 3$.) *C,* There was a small trend toward lower sequence stereotypy in birds with greater damage to Area X ($R^2 = 0.165$, $p = 0.17$). The *larger circle* in *B* indicates two birds with the same values.

notes were significantly more variable but not longer than intervals of intact birds. The song repertoire of deaf animals contained on average more notes than that of LMAN-lesioned birds but less than that of intact or Area X–lesioned birds (Fig. 13*C*). The majority of notes (17 out of 22) had patterns of frequency modulations similar to those found in subsong of intact animals, where sounds are noisier and frequency modulations are less well defined (cf. Price, 1979).

Figure 13, *A* and *B,* compares the duration and duration variation of notes and intervals in the intact group and the three different treatment groups. Each particular injury led to its own characteristic song, as defined by a unique combination of note and interval duration and duration variability values that differed from those of intact adult birds. This was also true when notes or intervals and their associated variability were simultaneously compared for all four groups in a multivariate ANOVA (Wilks's λ F test, $p = 0.0001$).

Development and crystallization of song in early Area X– and early LMAN-lesioned males

The songs of our intact zebra finch males showed little change after day 90 (cf. Immelmann, 1969; Arnold, 1975). However, we saw sustained change after that age in the song of the early Area X–lesioned males. In them, for example, note morphology

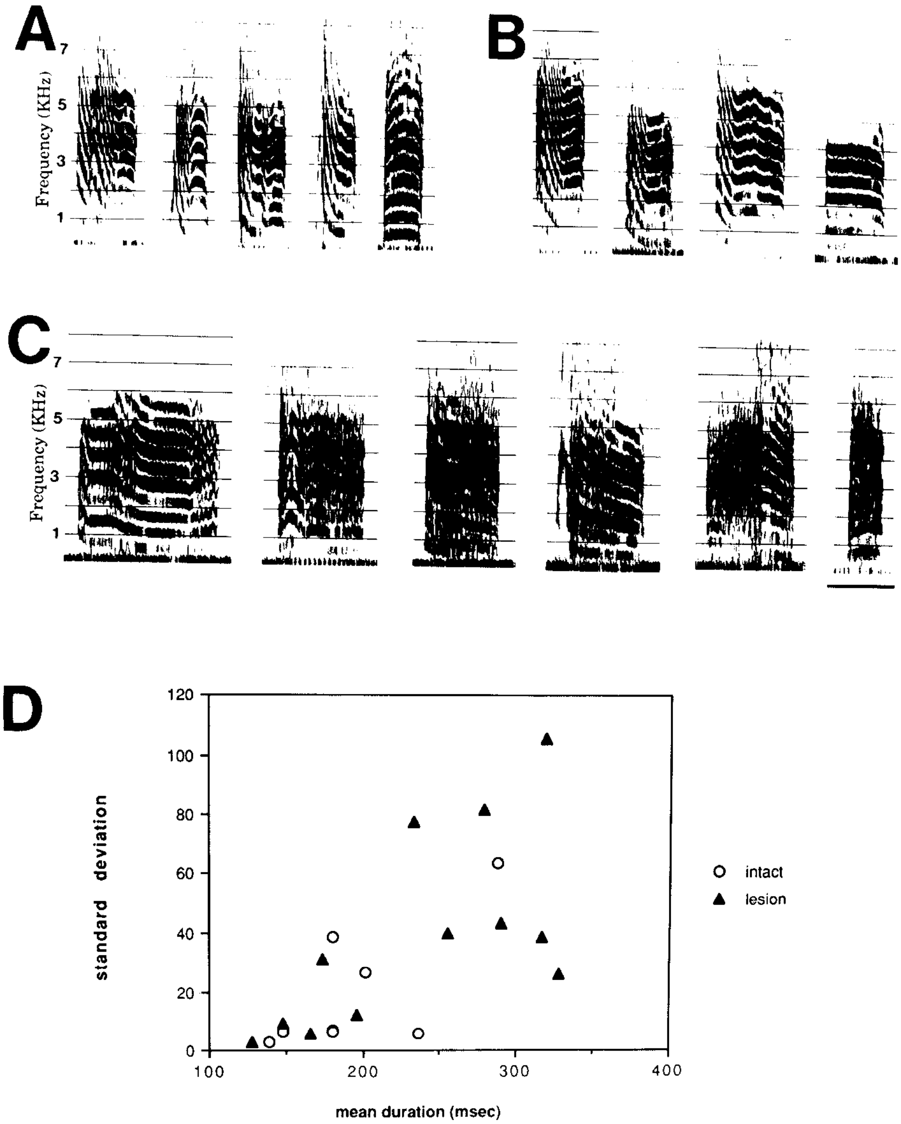

Figure 10. Representative distance calls of intact (*A*) and Area X–lesioned males (*B* and *C*) show that only a subset (*C*) of males that were lesioned had abnormal distance call morphology (*C*) and duration and duration variability (*D*). Scale bar, 250 msec.

and note order continued to change, but because of the variability in these parameters on any one day, this longer term change was difficult to quantify.

Early LMAN lesions had the opposite effect. Song of three birds with LMAN lesions was followed throughout development. Figure 14 shows song examples of one representative bird. Before the lesions, the animal was singing typical plastic song and exhibiting considerable variability in order and note morphology, including a few notes of adult morphology. By 1 d postsurgery, the song had shifted to a very stereotyped rendition

Table 3. Age at operation, lesion size, and song abnormalities of birds that received Area X lesions late in development

			Song					
			Note		Interval			
Bird band	% Lesioned	Age (d)	Duration	Variability	Duration	Variability	Morphology (abnormal notes/ total notes)	Sequence stereotypy
y27	73	64	+	+	NS	+	6/11	0.22
dg8	25	62	+	+	NS	+	6/7	0.18
lg112	55	61	+	−	NS	NS	2/5	0.83
lg101	100	66	NS	NS	NS	NS	0/10	0.69
dg109	56	62	NS	NS	NS	NS	2/7	0.69
dg108	48	70	NS	NS	NS	NS	1/6	0.83

Refer to Table 2 notes for details.

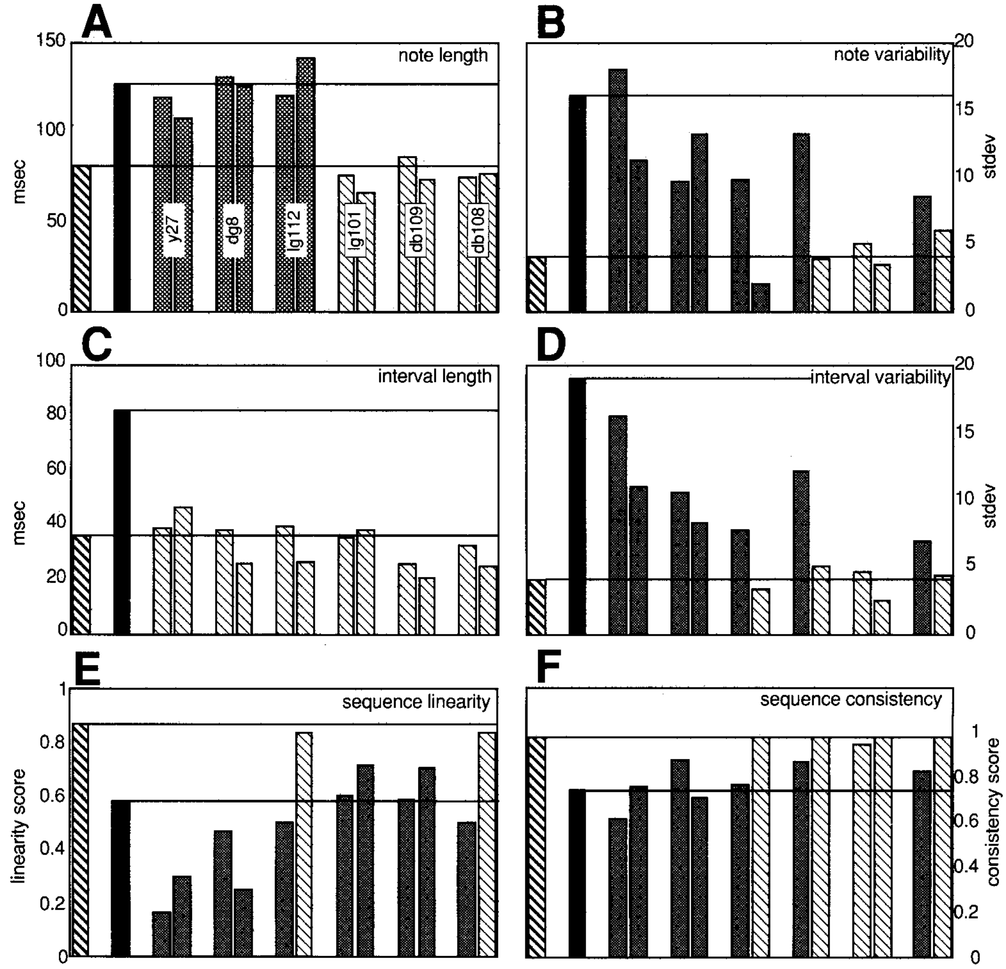

Figure 11. Songs that had already developed adultlike parameters were less susceptible to Area X damage late in development than songs whose notes were longer and characteristic of earlier song stages. Parameters of songs from six individuals before and after late Area X lesions are represented by their means of note length (*A*) and of its variability (*B*), means of interval length and its variability (*C* and *D*), and scores of sequence linearity (*E*) and sequence consistency (*F*). The identity of the six birds is shown in *A*. The *first bar of each doublet* represents the prelesion; the *second bar,* the postlesion value of the same bird. The data in each panel are compared to the mean of means of the intact (*first bar, thick cross-hatched fill*) and the early Area X–lesioned (*second bar, black fill*) populations. *Lightly cross-hatched fills* in *A–D* indicate that there was no significant difference between that bird's values and those of the intact birds (unpaired *t* test, $p > 0.05$). In *E* and *F*, *lightly cross-hatched fills* indicate values that are within the range of intact birds.

of the subset of prelesion notes that are discernible in the middle section of the middle sonogram of Figure 14*A*. The notes that were retained were not conspicuously different from other notes in the prelesion song. This bird and another bird with juvenile LMAN lesion did not show any song changes after 60 d of age. Two other birds were not recorded at that time but had arrived at their final song version by 84 d. Thus, while early Area X lesions seem to delay song crystallization, early LMAN lesions seem to hasten it.

Another difference in song development between birds with Area X or LMAN lesions is the retention of note morphologies that were part of the juveniles' repertoire before placement of the lesion. One animal that received an Area X lesion at 46 d had been recorded immediately prior to surgery, and a library of notes was generated from his song repertoire at that time. Among a majority of immature song elements, seven adult note types could also be identified. Of these, five were also found as almost identical copies in the adult repertoire of the lesioned bird's unoperated sibling (and cage mate). This suggests that had the experimental bird not received the lesion he would have incorporated these adult-type notes present at 46 d into his adult repertoire. After Area X lesion, his song did not contain those notes. In contrast, in LMAN-lesioned birds a subset of prelesion notes could be clearly identified after the operation (compare Fig. 14*A*, middle panel, with Fig. 14*B*).

Indirect effects of Area X lesions on HVC and LMAN

Electrolytic lesions of Area X might affect other song nuclei in a number of ways: Neurons in HVC that project to Area X could undergo retrograde degeneration after loss of their target. LMAN's function might be affected if fibers traveling from DLM to LMAN, and which course through Area X, were transected by the lesion (Bottjer et al., 1989). In both cases, cell loss in the affected nuclei could lead to shrinkage. We therefore measured nuclear volume of both HVC and LMAN in Area X–lesioned birds.

Animals that had received Area X lesions as juveniles had slightly but not significantly smaller HVCs, whereas LMAN of the early Area X–lesioned birds was significantly smaller than that of intact birds (Fig. 15). Interestingly, though, larger lesions of Area X did not have a consistently greater effect on LMAN size than smaller lesions of Area X: the mean LMAN volume (0.099 mm^3) of birds with Area X lesions greater than 75% was identical to the mean LMAN volume of birds with Area X lesions smaller than 30%.

To check if song quality was correlated with LMAN volume, overall song quality was considered normal when all six quantified parameters (note and interval length, note and interval variability, morphology, and stereotypy) fell within the intact distribution. Each parameter that was different from the intact

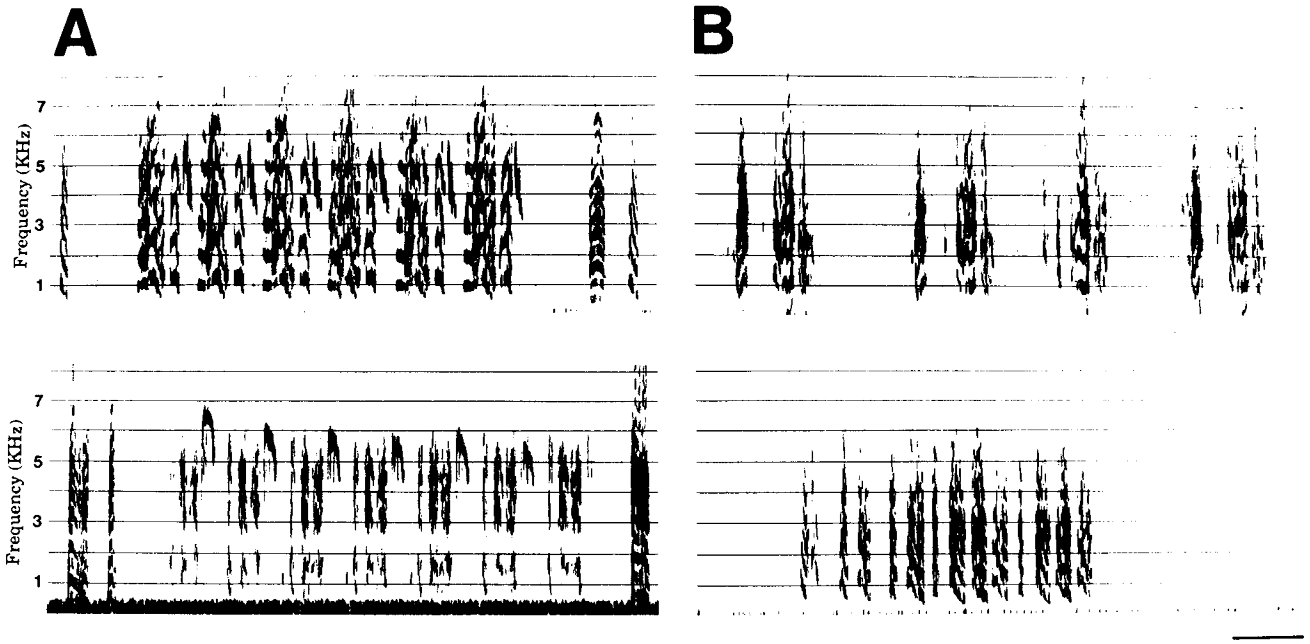

Figure 12. Song of four individuals that received LMAN lesions (*A*) or were deafened (*B*) as juveniles had characteristics such as note length and morphology that were typical for the injury incurred (see Results for details). *A, Top*: or/lg103, lesion at 43 d, 95% lesioned; *bottom*: lb/pk96, lesion at 40 d, 64% lesioned. *B, Top*: lb/y3, deafened at 29 d; *bottom*: lb/y53, deafened at 26 d. All sonograms shown were recorded when the birds were adult. Scale bar, 250 msec.

population was given a score of 1, resulting in a total score of 6 for the most abnormal song. Song quality of Area X–lesioned juveniles tended to be worse in those animals that had smaller LMAN volumes ($R^2 = 0.241$, $F = 5.078$, $p = 0.0386$), and so it is possible that part of the effect of early Area X lesions was mediated by the consequences of these lesions on LMAN.

Section of syringeal nerves

Both frequency modulation and temporal patterning are learned in intact song (Price, 1979). Overall temporal patterning of song persists following syringeal denervation, but frequency modulation is modified (Fig. 16*A*; cf. Williams et al., 1989; Simpson and Vicario, 1990). We wanted to see how syringeal denervation affected the adult song of birds that had received an early Area X lesion. As in intact birds, bilateral section of the tracheosyringeal nerve left the overall temporal pattern of song intact, but frequency modulation was affected (Fig. 16*B*). In addition, detailed analysis of note and interval length after nerve section in intact and Area X–lesioned males revealed a characteristic pattern. In both cases, all notes became longer and all intervals shorter (Fig. 17).

Discussion

Earlier reports have described the effects of early LMAN (Bottjer et al., 1984) and Area X (Sohrabji et al., 1990) lesions and of deafening (Price, 1979) on song development in the zebra finch. All three treatments interfered with the normal course of song acquisition in interesting ways. However, these studies were reported by different groups; the way behavioral deficits and extent of the lesions were assessed varied considerably, and the time window during which the manipulations were performed was wide (16–50 d). It was therefore difficult to relate these studies to each other.

The data reported here compare quantitatively the effects on song after early deafening and after lesions of both LMAN and Area X. This approach not only allowed us to substantiate observations of the previous reports but also enabled us to generate a new and specific hypothesis about the way auditory information and the recursive loop affect vocal learning.

Our results confirm the previous report of Sohrabji et al. (1990) that Area X is not necessary to maintain adult song production. It is, however, possible that Area X plays a perceptual role in adulthood, and we are in the process of investigating this possibility.

Our data also show that early lesions of Area X and LMAN have very different consequences on subsequent song development and on the characteristics of adult song. Whereas early Area X lesions led to protracted song instability (cf. Sohrabji et al., 1990), LMAN lesions led to an earlier development of stable song. In addition, the adult song developed by both types of birds differed in the number of notes included in the song—significantly fewer for LMAN-lesioned birds (cf. Bottjer et al., 1984) than for Area X–lesioned birds, who had on average the same amount of notes in their repertoire as intact birds. This latter finding is in conflict with the report of Sohrabji et al. (1990) that song of early Area X–lesioned birds never contained more than three distinct notes (however, song of their control birds also had less notes than the song from birds raised in this laboratory). This disparity could have a number of causes. (1) Birds in the Sohrabji et al. (1990) study were separated from their parents between 35 and 40 d and kept with siblings thereafter, while birds in this study were exposed to adult birds in an aviary environment until at least 90 d of age. Even though in intact birds exposure to adult song is not *necessary* after 35 d (Böhner, 1990), juveniles are susceptible to influences that can modify their song after that time (Slater et al., 1988). Con-

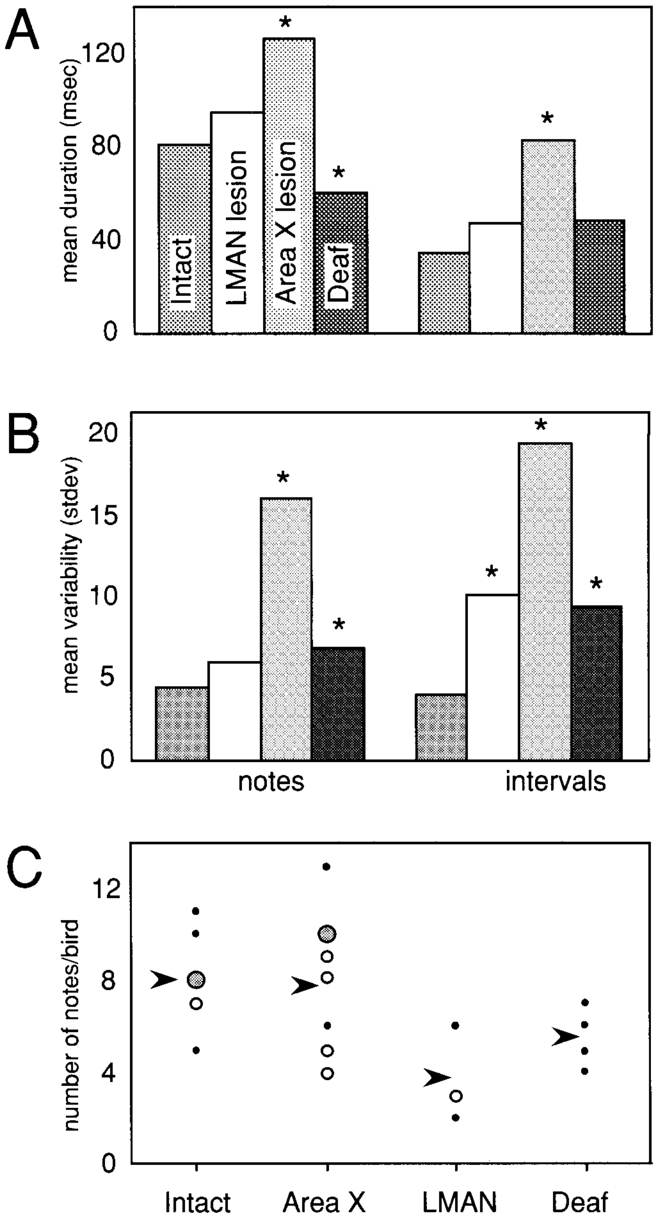

Figure 13. Note and interval length (*A*) and variability of those measures (*B*) in intact, early Area X–lesioned, LMAN-lesioned, and deafened animals. Shown are means of means. Number of notes : number of intervals were 64:44 (*Intact*), 14:12 (*LMAN*), 101:109 (*Area X*), 22:17 (*Deaf*). An *asterisk* indicates a significant deviation from the intact group at $p < 0.05$ (unpaired two-tailed Student's t test). *C,* Number of notes of each bird in the four treatment groups. *Arrowheads* indicate the group means. LMAN-lesioned birds ($p = 0.004$) and deaf birds ($p = 0.014$) both had significantly less notes than intact animals. LMAN-lesioned birds also had significantly ($p = 0.02$) less notes than Area X–lesioned birds. (Mann–Whitney U tests). *Large gray circles* indicate coincidence of three birds with the same number of notes; *medium open circles* indicate the coincidence of two birds with the same number of notes; *small solid circles* indicate single individuals.

tinued exposure to adult song models after lesioning brain areas involved in song acquisition clearly has the potential to affect subsequent song development, particularly since Area X continues to incorporate new neurons into its nucleus until at least day 55 (Nordeen and Nordeen, 1988). (2) Note numbers can also easily be underestimated, as we did in our preliminary report (Scharff and Nottebohm, 1989). This is because notes of distinct length and place within a motif can have very similar frequency patterns and stand out as individually distinct only after detailed analysis of many exemplars of one bird's song. Finally, (3) Sohrabji et al. (1990) used ibotenic acid as a lesion agent and only reported that lesion sizes exceeded 50%. Perhaps chemical lesioning results in more severe effects.

The LMAN and Area X early lesioned birds also diverged in the duration and variability of notes and intervals: Area X–lesioned animals had notes and intervals that were on average longer and more variable than was typical for intact birds, whereas LMAN-lesioned individuals deviated from normal birds only with respect to interval variability (Fig. 13).

The effects on song of these two lesion types are drastically different and cannot be sufficiently explained by the fact that LMAN is a smaller nucleus and was therefore more susceptible to complete destruction by the lesion. Even songs of birds whose Area X was almost completely lesioned (i.e., had between 10% and 20% of Area X left) did not at all resemble songs of LMAN-lesioned birds. For this reason, we also find it unlikely that the possibility of new neurons being added to Area X after lesion (but not LMAN; Nordeen and Nordeen, 1988) could compensate sufficiently for the damage in a way consistent with the observed behavioral differences. Song deficits of early Area X lesion could be seen as early as 10 d after the operation, too soon for neurogenesis to make a difference.

We conclude that the effects of either LMAN or Area X lesions cannot be interpreted as just the result of interrupting the circuit of the recursive loop. We suggest that the recursive loop plays two different roles: (1) modulator of the plasticity necessary for learning and (2) information channel necessary for learning. Part of the difference in the outcome following the two lesions could result from direct effects of LMAN on RA neurons. For example, hypothetical trophic factors released by LMAN innervation could play a role in the variability of motor programs—variability in note diversity, structure, duration, and sequencing. In the absence of LMAN, the number of note types would be reduced, notes would become more stereotyped, and sequencing would become more rigid, which is what we see. A similar effect could perhaps result from a replacement of synapses formed by LMAN neurons on RA cells (e.g., Canady et al., 1988) by synapses of a different origin, for example, from HVC; this replacement would alter the ratio of inputs on RA motor cells. These two effects need not be mutually exclusive. Whichever the mechanism, an increase in stereotypy following LMAN lesions could favor the retention of material learned up to the time of the lesion—or at least the retention of a subset of this material, as seems to be the case in LMAN-lesioned birds. Interestingly, a reduction in LMAN volume occurs as the bird makes the transition from the extreme variability of subsong to the more limited variability of plastic song (Bottjer et al., 1985; Korsia and Bottjer, 1989).

Removal of Area X need not interfere with LMAN's trophic or anatomical relations with RA, that is, with LMAN's putative role as enhancer of motor variability. However, removal of Area X may interrupt the flow of information necessary for learning. If so, this could explain why the song of early deafened and early Area X–lesioned birds shared some properties such as instability and poor syllable structure. Information that had been learned up to that time but had not yet manifested itself in its adult form did not develop further or deteriorated. This effect is particularly clear when comparing the preoperative and postoperative songs of birds that received Area X lesions late in vocal development: notes that were already well developed did not suffer from this lesion. Also consistent with this hy-

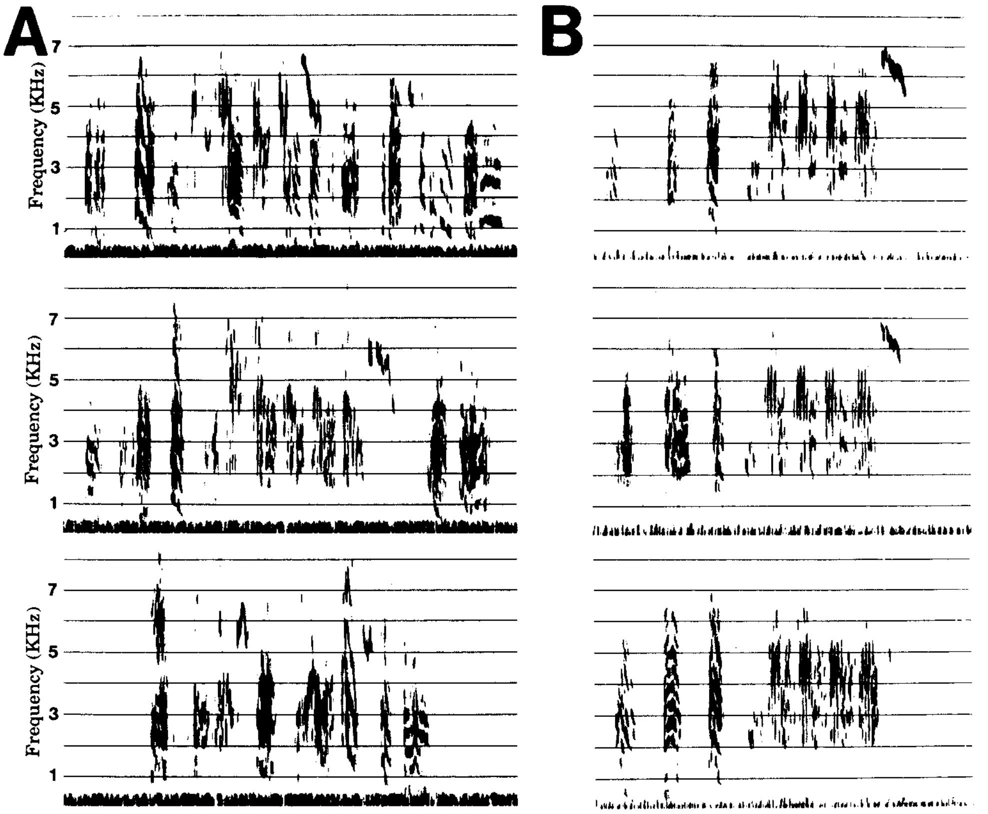

Figure 14. Effect of early LMAN lesion on song crystallization. *A,* Bird or/lg103 1 d before LMAN was lesioned at 43 d; *B,* 1 d after the lesion. Three different sonograms are shown from each recording session, illustrating the variability in note morphologies and ordering before LMAN lesion and the remarkable stereotypy one day postlesion (*B*). The postlesion song incorporated a sequence of notes from the prelesion song that is visible in the *middle section of the middle sonogram* in *A.*

pothesis is the fact that the note structure most representative of early Area X–lesioned birds is not unlike that of notes typically encountered in subsong.

The effects of early deafening on note structure and variability observed in this study coincide with the earlier findings (Price, 1979). It is, however, difficult to compare data from these two studies because Price (1979) pooled data from birds deafened between 16 and 43 d, and it is likely that deafening over such a wide range of ages produced more heterogeneous effects than the ones observed in our birds that were all deafened around 28 d. Comparing deafened animals to intact adults, Price (1979) found notes to be of the same duration as adults, but intervals were longer. In our set of experiments, early deafening resembled the effect of early Area X lesions in terms of note frequency patterns that create the noisy appearance of notes on sonograms, but most closely matched the effects of LMAN lesions in terms of interval length as well as interval and note variability. In addition, early deafening led to the development of significantly shorter notes than found in intact, LMAN-lesioned, or Area X–lesioned birds.

If the effect of interrupting the recursive loop were comparable to deafening, then deafening and early Area X lesions should lead to a similar kind of adult song because Area X lesions need not affect the anatomical or trophic relations that may occur between LMAN and RA, as suggested above. However, early deafening and early Area X lesions did not have identical consequences on song. This is probably not due to the fact that deafening occurred at a slightly earlier age than lesioning of Area X. Four animals were deafened or received Area X lesions only a few days apart at around the time that the first vocalizations can also be heard [i.e., deafened at 28 or 29 d; Area X lesioned at 31 d; first vocalizations can start as early as 25 d and may do so earlier (cited in Slater et al., 1988)]. The song of the earliest Area X–lesioned birds was different from that of the deafened birds but very similar to that of the rest of the birds in that treatment group (compare Fig. 5*B* with top of Fig. 12*B*).

Thus, the difference between the song of the Area X and deafened birds suggests that even though in both cases information flow necessary for learning has been interrupted there must also be some other factors at play.

Even when adult song is not the result of successful matching of a tutor's model, as in Area X–lesioned birds, the central program for singing involves nonetheless participation of the syringeal innervation usually associated with learning (Simpson and Vicario, 1990). This is shown by the fact that tracheosy-

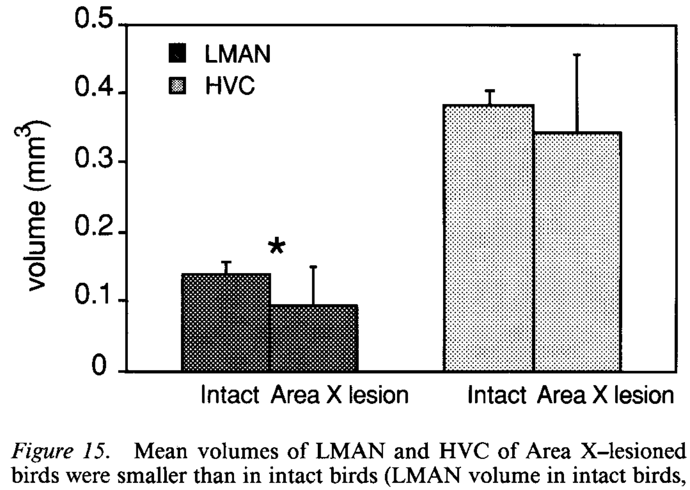

Figure 15. Mean volumes of LMAN and HVC of Area X–lesioned birds were smaller than in intact birds (LMAN volume in intact birds, $n = 8$; in Area X–lesioned birds, $n = 9$; HVC volumes in intact birds, $n = 7$; in Area X–lesioned birds, $n = 8$). The *asterisk* indicates $p < 0.05$, Mann–Whitney U test, $U = 0$; error bars indicate 1 SD.

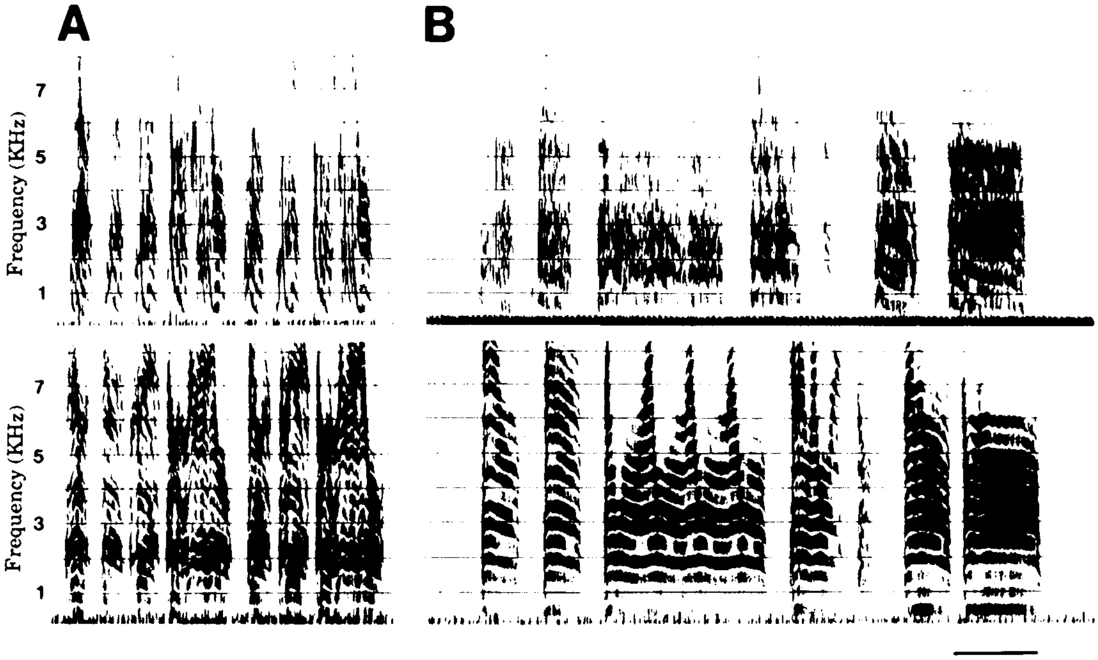

Figure 16. Effect of hypoglossal nerve section on note morphology. Sonograms of song from one otherwise intact bird (*A*) and from an Area X-lesioned bird (*B*). *Top panels* are representative examples before hypoglossal nerve section; *bottom panels* illustrate that song after nerve cut lost much of its frequency characteristics but preserved the overall temporal pattern. Scale bar, 250 msec.

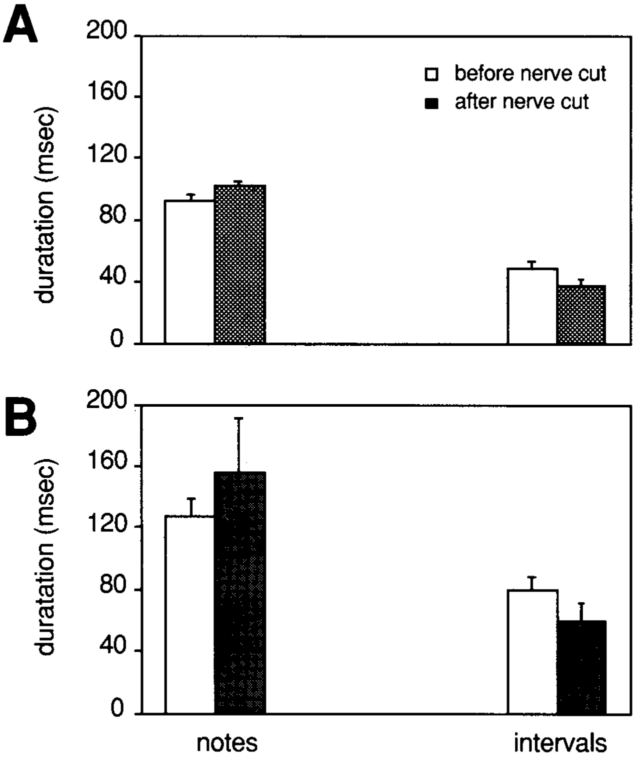

Figure 17. Effect of hypoglossal nerve section on note and interval length. *A*, Mean of note ($n = 31$) and interval ($n = 24$) means before (*white bars*) and after (*black bars*) nerve section in otherwise intact adult subjects. Notes became longer by an average of 7% (between 2% and 20%); intervals shortened by an average of 35% (between 9% and 64%). Significant at $p = 0.01$ (Wilcoxon matched-pairs, signed-rank test). *B*, The same pattern was seen after nerve section in adult animals that had previously received Area X lesions as juveniles (notes, $n = 11$; interval, $n = 8$; error bars indicate 1 SD).

ringeal nerve section modified frequency modulation in the early Area X–lesioned birds. Thus, it seems that the program for song development mandates what circuitry will be used whether or not the bird can integrate the information normally required for learning.

Auditory information reaches RA (Williams and Nottebohm, 1985). RA receives input from HVC and from LMAN. Auditory responses can be recorded from RA soon after they occur in HVC and before they appear in Area X, suggesting a direct HVC–RA auditory connection (Williams, 1989). Auditory responses can also be recorded from Area X and LMAN (Doupe and Konishi, 1989; Williams, 1989). We suggest that for song learning to take place auditory information must reach RA both via the HVC–RA efferent pathway and via the recursive loop. Early destruction of Area X would block auditory access to the recursive loop but would not eliminate HVC to RA auditory influences on song. Deafening would deprive both routes to RA of any access to auditory input. This may explain why deafening and Area X lesions had a different behavioral outcome. Though our emphasis here has been on the routes used by the auditory input to the efferent pathway for song, motor activity may also travel through the recursive loop and play a role in vocal learning.

We have tried to interpret the results of early deafening or early lesions of LMAN and Area X in ways consistent with our knowledge of anatomical, physiological, and behavioral facts. Clearly, LMAN and Area X play different roles in song acquisition, even though they are part of the same pathway.

References

Alvarez-Buylla A, Vicario DS (1988) Simple microcomputer system for mapping tissue sections with the light microscope. J Neurosci Methods 25:165–173.

Arnold AP (1975) The effects of castration on song development in zebra finches (*Poephila guttata*). J Exp Zool 191:261–278.

Böhner J (1983) Song learning in the zebra finch (*Taeniopygia guttata*): selectivity in the choice of a tutor and accuracy of song copies. Anim Behav 31:231–237.

Böhner J (1990) Early acquisition of song in the zebra finch, *Taeniopygia guttata*. Anim Behav 39:369–374.

Bottjer SW, Miesner EA, Arnold AP (1984) Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science 224:901–902.

Bottjer SW, Glaessner SL, Arnold AP (1985) Ontogeny of brain nuclei controlling song learning and behavior in zebra finches. J Neurosci 5:1556–1562.

Bottjer SW, Halsema KA, Brown SA, Miesner EA (1989) Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. J Comp Neurol 279:312–326.

Canady RA, Burd GD, DeVoogd TJ, Nottebohm F (1988) Effect of testosterone on input received by an identified neuron type of the canary song system: a Golgi/electron microscopy/degeneration study. J Neurosci 8:3770–3784.

Clayton NS (1987) Song tutor choice in zebra finches. Anim Behav 35:714–721.

Doupe AJ, Konishi M (1989) Auditory properties of song nuclei of estrildid finches. Soc Neurosci Abstr 15:347.

Eales LA (1985) Song learning in zebra finches: some effects of song model availability on what is learnt and when. Anim Behav 33:1293–1300.

Gurland J, Tripathi RC (1971) A simple approximation for unbiased estimation of the standard deviation. Am Stat 25:30–32.

Hall MF (1962) Evolutionary aspects of estrildid song. Symp Zool Soc Lond 8:37–55.

Immelmann K (1968) Zur biologischen Bedeutung des Estrildidengesanges. J Ornithol 109:284–299.

Immelmann K (1969) Song development in the zebra finch and other estrildid finches. In: Bird vocalisations (Hinde RA, ed), pp 61–74. London: Cambridge UP.

Katz LC, Gurney ME (1981) Auditory responses in the zebra finch's motor system for song. Brain Res 211:192–197.

Kelley DB, Nottebohm F (1979) Projections of a telencephalic auditory nucleus—field L—in the canary. J Comp Neurol 183:455–470.

Konishi M (1964) Effects of deafening on song development in two species of juncos. Condor 66:85–102.

Korsia S, Bottjer SW (1989) Developmental changes in the cellular composition of a brain nucleus involved in song learning in zebra finches. Neuron 3:451–460.

Nordeen EJ, Nordeen KW (1988) Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. J Neurosci 8:2869–2874.

Nottebohm F (1987) Birdsong. In: Encyclopedia of neuroscience (Adelman G, ed), pp 133–136. Boston: Birkhauser.

Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary, *Serinus canaria*. J Comp Neurol 165:457–486.

Okuhata S, Saito N (1987) Synaptic connections of thalamo-cerebral vocal nuclei of the canary. Brain Res Bull 18:35–44.

Price PH (1979) Developmental determinants of structure in zebra finch song. J Comp Physiol Psychol 93:260–277.

Scharff C, Nottebohm F (1989) Neural mechanisms of behavior. In: Proceedings of the 2nd international congress of neuroethology (Erber J, Menzel R, Pflüger H-J, Todt D, eds), pp 122. Stuttgart: Thieme.

Simpson HB, Vicario DS (1990) Brain pathways for learned and unlearned vocalizations differ in zebra finches. J Neurosci 10:1541–1556.

Slater PJB, Eales LA, Clayton NS (1988) Song learning in zebra finches (*Taeniopygia guttata*): progress and prospects. In: Advances in the study of behaviour, Vol 18 (Rosenblatt J, Beer C, Busnel M-C, Slater PJB, eds), pp 1–32. San Diego: Academic.

Sohrabji F, Nordeen EJ, Nordeen KW (1990) Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav Neural Biol 53:51–63.

Sossinka R, Böhner J (1980) Song types in the zebra finch *Poephila guttata castanotis*. Z Tierpsychol 53:123–132.

Stokes TM, Leonard CM, Nottebohm F (1974) The telencephalon, diencephalon, and mesencephalon of the canary, *Serinus canaria*, in stereotaxic coordinates. J Comp Neurol 156:337–374.

Williams H (1989) Multiple representations and auditory–motor interactions in the avian song system. Ann NY Acad Sci 563:148–164.

Williams H (1990) Models for song learning in zebra finches: fathers or others? Anim Behav 39:745–757.

Williams H, Nottebohm F (1985) Auditory responses in avian vocal motor neurons: a motor theory for song perception in birds. Science 229:279–282.

Williams H, Cynx J, Nottebohm F (1989) Timbre control in zebra finch (*Taeniopygia guttata*) song syllables. J Comp Psychol 103:366–380.

Zann R (1985) Ontogeny of the zebra finch distance call. I. Effects of cross-fostering to bengalese finches. Z Tierpsychol 68:1–23.