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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

1. Behavioral paradigms have been developed that yield quantifiable, reliable results for testing the discriminability of two auditory stimuli (operant go-nogo) and individuals' preferences between two stimuli (two-speaker choice test). The copulation solicitation respons is not reliable.

2. Zebra finches can learn to produce and discriminate variants in the 'timbre' of song syllables. Adult males learn a discrimination between two similar songs more quickly when one of those songs is their own.

3. Auditory responses have been recorded, measured, and cataloged in all the forebrain nuclei with connections to the song motor system. The latencies may give indications of how this auditory information is processed.

4. Deafening studies had led to the conclusion that vocal plasticity ceased at sexual maturity in 'closed-ended' song learners. This is not so: when a hearing male's song is altered by cutting the vocal motor nerves, a limited form of plasticity in production is seen.

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Final Annual Technical[1] Report

AFOSR-86-0336
1 Sep 87 - 31 Aug 88

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Title: Motor Theory of Auditory Perception

Research on this project that was funded by the AFOSR has been completed, although work continues on issues that have arisen out of the findings made during the study.

During the past year[2], efforts have concentrated upon:

1) Defining behavioral tests of auditory discrimination
2) Using these tests to determine which parameters of bird song are discriminated
3) Evaluating the role in these discriminations of the vocal motor areas in the forebrain
4) Determining the presence and latency of auditory activity in the forebrain centers associated with vocalization.

These areas of concentration focus directly upon a critical question in the evaluation of motor theories of perception: do complicated communication signals from conspecifics require special processing in the brain, or are acoustic analyses of these signals sufficient for extracting the information encoded in these signals?

Behavioral measures of auditory perception.

Although behavioral response measures of perception have proven to be difficult to define, we (Dr. Jeffrey Cynx, Dr. Stephen J. Clark, and the P.I.) now have three sets of protocols in operation, and a fourth shows potential.

Copulation solicitation response by females.

This method for measuring females' behavioral responses to the songs of different

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[1] Financial and Patent Reports are being forwarded under separate cover by officials of The Rockefeller University.

[2] Please refer to the annual technical report for AFOSR-86-0336 covering the period 1-Sep-86 to 31-Aug-87 for a complete report on the first year of work covered under this contract. Results and publications listed in that report are not repeated here.

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males was developed by Dr. William Searcy at the Rockefeller University Field Research Center (Searcy and Marler, 1981). Females are implanted with Estradiol and isolated from contact with other birds, both of which treatments increase sexual responsiveness. Females are then presented with a variety of song types, and the duration and intensity of any copulation solicitation response displays is evaluated. This method has several drawbacks:

- Comparison of male and female responses is problematic, since no equivalent response is shown by males. Song playbacks to males generally result in aggressive displays, and the difference in context of the two responses complicates interpretation.

- Levels of hormones may affect responsiveness. A female with a higher or lower hormone might then skew the results dramatically. Controlling for hormone levels requires extensive blood sampling and testing using RIAs; this is expensive and time-consuming.

- We find that data obtained using this method with female zebra finches are not reliable. A large effect on the first day of testing disappears or reverses on the second day. Habituation seems to be a severe problem; using additional animals with controls for order of presentation of the stimuli is necessary, and when more than two stimuli are being used on a limited number of animals (animals with known fathers, brothers, and experience require many man-hours to obtain) collecting good data is problematic at best.

Two-speaker approach test.

In this paradigm (see Miller, 1979a,b), a single zebra finch is placed in a small enclosure inside a long, narrow cage. Two speakers play the two stimulus sounds, one at either end of the test apparatus. After a short period of habituation to the apparatus and exposure to the test stimuli, the door of the small cage is opened by remote control and the subject is free to approach either speaker. Preliminary data using conspecific and extra-specific song show that choices of approach stimulus are strong, quantifiable, and reliable over several days of re-testing. The advantage of this method is that it is quick, requires no hormonal treatments, and the behaviors measured have no strong sexual valence. Since we need to be able to compare males' responses to acoustic stimuli to those of females, this method shows great promise. Work along these lines is continuing, being performed by Dr. Stephen J. Clark at Rockefeller University, in consultation with the P.I.

Operant conditioning: go-nogo.

Zebra finches can be trained to perform go-nogo operant responses easily, as they are active and readily investigate all aspects of a new environment. In the paradigm Dr. Jeffrey Cynx and the P.I. have used, the bird starts a trial by hopping onto a perch and breaking an infrared beam. This is followed by the presentation of an auditory stimulus. If this is a 'go' stimulus, the bird must move to a perch in front of a food hopper within three seconds to receive access to that hopper. Otherwise ('nogo' stimulus), the bird remains

on the perch for three seconds and is then given access to food[3]. We have found that, for many types of auditory stimuli and discriminations, zebra finches can learn to perform to criterion within a few days of initial training. Nevertheless, some reservations about the method remain: even the fastest learners take a few thousand trials to pick up discriminations between natural stimuli which would be salient and important in their environment - and, in that context, would be learned in within only a few trials. Perhaps the large number of learning trials is necessary because of a sensory/motor mismatch (food reward to an auditory stimulus). It would be interesting to try a visual or auditory reward - such as the playback of a song or a female's calls, or the presentation of a picture or video of a conspecific. Zebra finches are highly social animals, and an isolated animal always moves to join a group; to an individual isolated in an operant chamber, sight or sound of a conspecific may well be the most potent reinforcer possible.

Auditory discrimination of conspecific signals.

We have attempted to define characteristics of zebra finch song that seem, to the human ear or brain, subtle enough that they might require special processing by the neural centers that analyze conspecific song. This approach has led us to two attacks on the problem, one of which involves analysis at the level of the single syllable, and the other at the level of an entire song.

'Timbre' learning, production, and discrimination in zebra finch song syllables.

We have used the word 'timbre' to define the pattern of emphasis and suppression of the harmonics in a single syllable. Many zebra finch song syllables consist of a 30-150 ms note in the form of a series of harmonics which show little frequency modulation. However, the relative amplitude of each harmonic can vary widely, over a range of up to 50 dB relative to the loudest harmonic within a syllable.

In research reported at the 1988 Society for Neuroscience Meeting in Toronto and in two papers which are in press (see list of publications and Appendices I and II), Drs. Cynx, Nottebohm, and the P.I. found that:

- An individual zebra finches can produce a wide range of timbre variants within his song.
- A population of 12 zebra finch songs contains many timbre variants, some of which are more common than others. Timbre is not related to fundamental frequency or to the modulation patterns seen in the syllable.
- The timbre of a syllable within a male's song is constant over an extended period of time, and probably throughout the individual's life; it can then be said to be a fixed characteristic of the song syllable.
- Timbre is learned. Young males copy the timbre of the syllables they acquire from a

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[3]A left-right choice between perches is learned only after many more trials than are needed to acquire a discrimination using a go-nogo paradigm.

model song sung by an adult male.
- The central nervous system controls the timbre of zebra finch song syllables; this control seems to be exerted at the level of the syrinx (the avian vocal organ).
- Zebra finches (and canaries) can learn to discriminate between two timbre variants of the same syllable.
- This discrimination depends upon both a) the sound energy levels at the frequency which varies between the two syllables with differing timbre and b) the harmonic nature of the energy in surrounding frequencies.

Discrimination of complete songs.

Following the argument of Margoliash and Konishi (1985), that the bird's own song has special salience as an auditory stimulus for the neurons of the song motor system, Drs. Cynx, Nottebohm, and the P.I. decided to test zebra finch males' discriminations of pairs of songs. Song pairs were chosen so that both songs had similar temporal structure and were composed of similar notes; in short, the two songs were as nearly identical as could be found among songs from the population on hand and on record from previous studies. Zebra finches were then trained up on a go-nogo discrimination in which a pair of similar songs contributed the 'go' and 'nogo' stimuli. We found that zebra finch males that were given this difficult discrimination learned it much more quickly (half the number of trials) when one of the two songs was their own. When neither of the two similar songs was the bird's own song, the discrimination was far more difficult. This result points to a possible lead in the attempt to define auditory stimuli which are difficult to discriminate without the aid of a special processor - such as the one which is proposed by the motor theory of song perception.

The role of forebrain vocal production centers in the discrimination of conspecific signals.

Given that we had described two possible types of auditory stimuli which seemed to humans to be difficult to process and which could be discriminated by zebra finches, the next step was to investigate the effect of lesioning stations in the song motor system upon the performance of these auditory discriminations.

Initially, the results were discouraging. We lesioned HVc, a song system nucleus (see Figure 1), as well as some of the surrounding tissue (possibly including small portions of primary auditory cortex) and re-tested the birds on the learned discrimination. No deficits in performance were seen

Later, however, we tried the effect of lesioning HVc before training the birds to perform the discrimination. In this case, a severe deficit in learning the discrimination was found. These preliminary results indicate that HVc is required to learn a complex auditory discrmination such as that between timbre variants or two similar songs - but is not needed for the maintenance of that discrimination. We do not yet know how to interpret this finding.

Auditory activity in forebrain song system nuclei.

Auditory activity is found throughout the song system (Figure 2). Neurons in the nuclei of the efferent branch of the song system (the pathway leading directly to the motor neurons; see Fig. 1) as well as the recursive branch (nuclei connecting HVc indirectly to RA; see Fig. 1) respond to acoustic stimuli. This auditory responsiveness extends to the level of the motor neurons innervating the vocal organ (Williams and Nottebohm, 1985). Since both branches of the song system are necessary, albeit at different stages of development, for the production of vocalizations, auditory functions

can be said to coexist with motor functions in the neural circuitry for vocalization. Two possiblities, which are not necessarily exclusive, come to mind when functions for this auditory activity in the motor system are proposed.

1. A motor theory of perception, as outlined above (and formulated for human speech perception by Liberman et al. (1967)): complex conspecific signals are analyzed by comparing them to the motor gestures the animals would have used to produce similar sounds. The extracted sequence of motor gestures defines the information in the signal.

2. Auditory-motor comparisons are essential for song learning, as young birds learn to sing by imitating a song model which is first tested against an innate representation of song, stored in memory, and then compared to the young bird's own vocalizations. This process must require comparison of auditory and motor representations of song at several stages of the learning process, and so might account for the extensive interdigitation of auditory and motor functions seen in the avian brain's song system.

What we know about the anatomy of the song system and the auditory information represented within that system gives some hints as to the functions that auditory activity may have within the motor system. Efferents from field L, the primary auditory projection to forebrain, are found in an area immediately adjacent to RA as well as in the shelf underlying HVc (Kelley and Nottebohm, 1979). Lesioning HVc or RA eliminates auditory responses in the vocal motor neurons(Williams and Nottebohm, 1985). Any auditory inputs RA receives independently of those in the projection from HVc are not effective in activating the pathway to the vocal motor neurons. Auditory inputs to HVc are then the primary source of the auditory activity in the efferent branch of the song system.

Since HVc neurons project to area X as well as to RA, the auditory activity seen in the song system's recursive loop may also be dependent upon auditory inputs to HVc. Another possible source of auditory inputs to area X and lateral MAN are the secondary auditory projections to the anterior portion of the forebrain (Bonke et al., 1979). Auditory responses in the descending pathway might also activate reafferents which could in turn be relayed to the recursive loop, perhaps through the thalamic nucleus DLM. Regardless of which of these potential sources of auditory inputs to the recursive loop are responsible for the auditory activity seen in lateral MAN, that activity can be relayed back to RA. The auditory response seen in lateral MAN has a longer latency than that in RA (see Figure 2), indicating that auditory input from MAN cannot contribute to the auditory responses seen in RA and the vocal motor neurons. However, two other possible sources of auditory input to RA (from field L and from lateral MAN) might play a role in comparisons among different representations of song.

These observations indicate that the circuitry cannot subserve a model of simple matching of various auditory and motor representations of song. However, song learning may require a more complex circuit than that simple model, and the presence of auditory neurons within HVc which respond to the bird's own song before song learning is completed (Volman, 1987) would indicate that song learning does indeed use the auditory responses in the song system as a substrate.

Lesion studies have shown that the song system nuclei in the efferent pathway (NIf, HVc, RA) are necessary for the production of song in adult zebra finches (McCasland, 1987). In contrast, lesioning the lateral MAN, a nucleus of the recursive branch, only affects song learning, and not song production in adults (Bottjer et al., 1984). This would indicate a partitioning of roles for auditory inputs as well as for motor activity in the two branches of the song system circuitry. Although we do not yet know much about the response properties of sound-sensitive neurons within the nuclei of the recursive loop, the P.I. has found that these neurons do respond to song stimuli as well as to pure tones and white noise in anesthetized animals (unpublished data). Comparative studies of auditory response selectivity in the two branches of the song system would help in elucidating their potentially different roles in analyzing auditory information. Since HVc lies at the junction of the two song system branches and contains two separate sets of projections neurons (to RA, in the efferent branch, and area X, in the recursive branch - see Fig. 1), the best way to approach this problem might be a study of response properties of these two sets of HVc neurons. The preliminary results cited above, indicating that HVc is needed for the learning but not the maintenance of difficult auditory discriminations, imply that HVc is indeed important for some forms of auditory processing - and it is also an easily accessible brain area.

Plasticity in an adult closed-ended learner.

A serendipitous finding made while studying the effect of vocal organ denervation upon timbre (see above and appendix I) may prove to lead to a strong line of research. It has been thought that avian species designated 'closed-ended' song learners complete song learning early in life and thereafter proceed to sing using a 'motor tape', or pattern of muscle activity that is not affected by auditory feedback. Zebra finches are thought to be a classic example of this type of learning; deafening does not affect song (Price, 1979), in contrast to the severe deficits seen in canary song a few weeks after deafening (Marler and Waser, 1979).

Adult zebra finch males had both their tracheosyringeal nerves cut in the course of studying timbre production, and, since no further work on their brains was planned, were allowed to survive for extended periods and recorded periodically. As is shown in Figure 3, changes in the song could be seen. Two types of changes occurred. The first, observed immediately after nerve section, was a homogenizing of syllable morphology, with each syllable becoming some variant of a harmonic series, with little of the dramatic frequency modulations or filtering which can be seen in the intact animal. The length of syllables, their amplitude profile, and the order of delivery remained constant after the nerve section, which allowed an accurate comparison of syllables within the two songs. The second type of change was a consistent dropping of four syllables from the center of the song; these syllables were not replaced by silence, but the beginning and end of the song were sung in succession. In effect, the syllables were 'spliced' out of the song and consequently of the 'motor tape' as well.

This finding is interesting because it contradicts the theory that auditory feedback has no access to the vocal motor program. In these birds, the auditory system is intact, as is the motor system (except for the axons leading to the vocal organ). After nerve section, the song produced is abnormal, and the bird hears that abnormality and attempts to correct it. It is suggestive that the syllables which were 'spliced out' of the song were those that had the greatest frequency modulation in the intact animal and so were the most difficult to reproduce when the syrinx was denervated.

This result opens the door to an interesting series of questions on how the bird's own song is perceived during song learning and after that process is completed, and as to whether changes that occur during 'crystallization', or the end of the learning period, are as complete as had been thought - or whether there are two systems for song learning and perception, one dealing with syllable morphology and the other with syllable order and timing.

Preliminary conclusions.

Although there has been no striking breakthrough in assessing the viability of motor theories for perception during the course of this study, there have been advances on a number of fronts. We now know that the signal properties of avian vocalizations are extremely complex, to the level that a special processing system for conspecific auditory signals may be necessary. We also know that the vocal motor system seems to be implicated in learning discriminations between complex conspecific sounds, and that critical periods in vocal learning may not have the monolithic rigidity that was once thought to exist.

Prospects for the future are good; work continues on the avenues opened during the past two years, and the problem has become more circumscribed. We now know where not to look, and have some indications of where we should direct our efforts.

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Williams, H., & F. Nottebohm. 1985. Auditory responses in avian vocal motor neurons: a motor theory for song perception. **Science.** **229** :279-282.

Publications

1. Williams, H., Cynx, J., & Nottebohm, F. (in press) "Timbre control in zebra finch song syllables" **J. Comp. Psychol.**

2. Cynx, J., Williams, H., & Nottebohm, F. (in press) "Timbre discrimination in zebra finch song syllables" **J. Comp. Psychol.**

3. Williams, H. "Bird Song" to appear in Cognitive Neuroscience, R. Kesner and D. Olton, eds.

4. Williams, H. "Multiple Representations and Auditory-Motor Interactions in the Avian Song System" to appear in Modulation of Defined Neural Circuits R. Schoenfeld, M. Davis, and B. Jacobs, eds.

Preprints of (1) and (2) are included in this report as Appendices (I) and (II). These represent unrevised editions of the papers that have been accepted, subject to revisions, by the Journal of Comparative Psychology.

Summary of expenditures under the current budget period
(1-Sep-87 to 31-Aug-88)

	Approved Budget	Expenditures	Balance
Direct costs	41,664	41,664	0
Indirect costs	28,123	28,123	0
Totals	69,787	69,787	0

Personnel engaged on project

Principal Investigator:
Dr. Heather Williams   Assistant Professor

Colleagues in the Nottebohm laboratory at Rockefeller University with whom collaborative research has been undertaken:
Dr. Jeffrey Cynx   Postdoctoral Fellow
Dr. Stephen Clark   Postdoctoral Fellow
Robert Morrison   Doctoral Student
Andrea DeMajewski   Undergraduate Assistant

Vertebrate Animals:
Zebra finch, *Poephila guttata.*
Canary, *Serinus canaria.*

I certify that the statements herein are true and complete to the best of my knowledge.
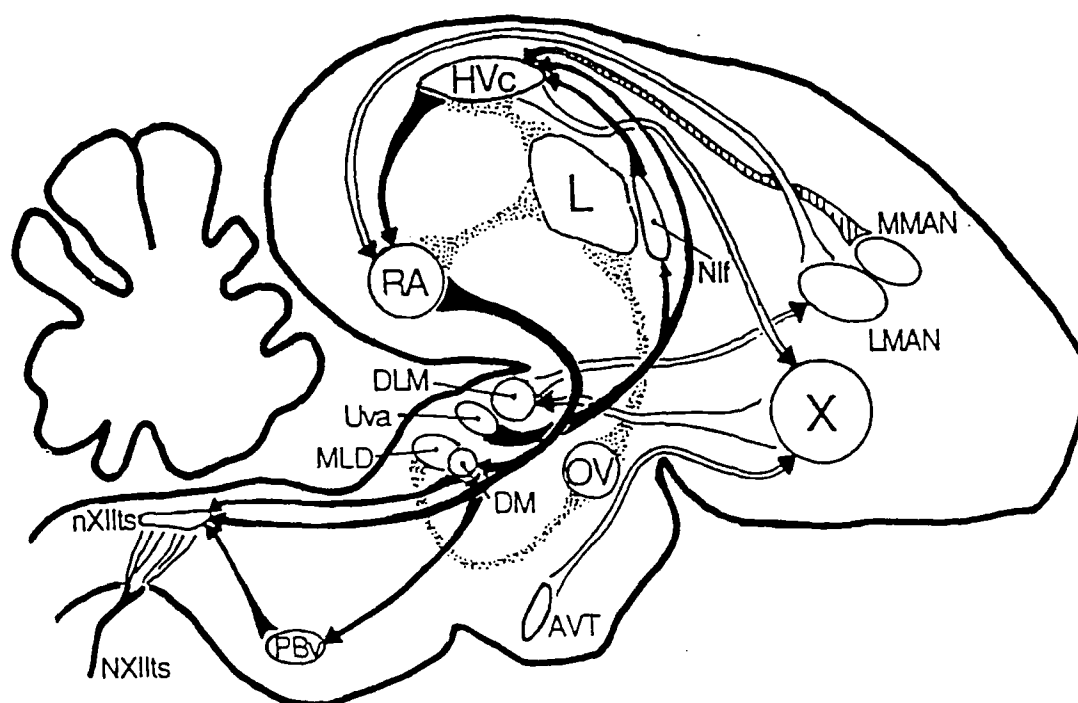
Heather Williams   2 Dec 88

Figure 1. **The avian song system.**

A composite sagittal section showing the nuclei of the song system and their connections. The efferent branch of the song system is shown with filled arrows, the recursive branch with open arrows, and the ascending auditory pathway with stippling.

AVT = Area ventralis of Tsai; DLM = the medial portion of the dorso-lateral thalamic nucleus; DM = the dorso-medial portion of the intercollicular nucleus (ICo); HVc = the high vocal center (also, but improperly, called hyperstriatum ventralis pars caudalis); L = field L, the primary telencephalic auditory projection; LMAN = the lateral portion of the magnocellular nucleus of the anterior neostriatum; MMAN = the medial portion of the magnocellular nucleus of the anterior neostriatum; MLD = the inferior colliculus; NIf = the interface nucleus; OV = nucleus ovoidalis, the thalamic auditory relay nucleus; nXIIts = the tracheosyringeal portion of the hypoglossal nucleus; NXIIts = the tracheosyringeal portion of the hypoglossal nerve, which innervates the muscles of the vocal organ; PBv = the ventral parabrachial nucleus; RA = nucleus robustus of the archistriatum; Uva = nucleus uvaeiormis of the thalamus; X = area X.
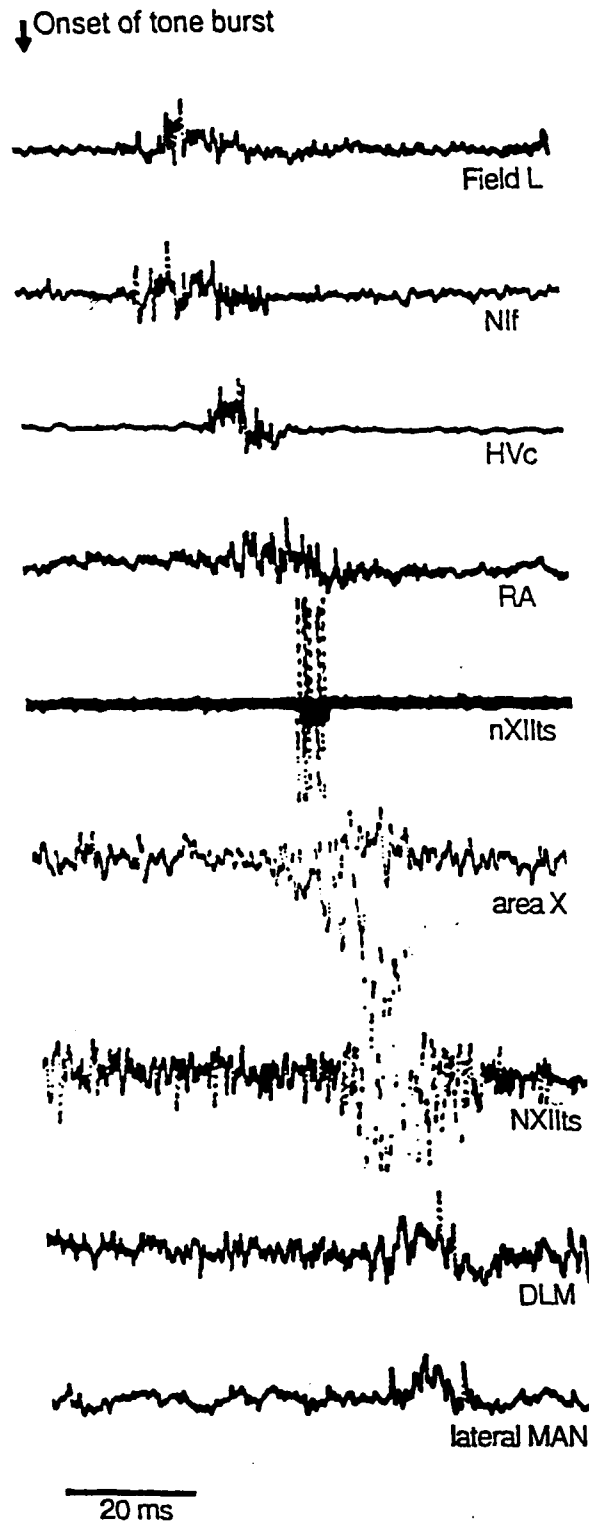
Figure 2.   Auditory responses in the song system.

Each trace represents the response to a tone burst as recorded extracellularly within a song system nucleus (the traces are labeled with the recording site; refer to Figure 3 for the location and connections of the nuclei). The recordings are arranged in the order of increasing latency from the stimulus onset.   (Note: these recordings were not all made in the same bird, and a small amount - up to 5 ms - of variability in absolute timing is possible among birds. However, the data shown here are representative of the relative latencies seen in all recordings).
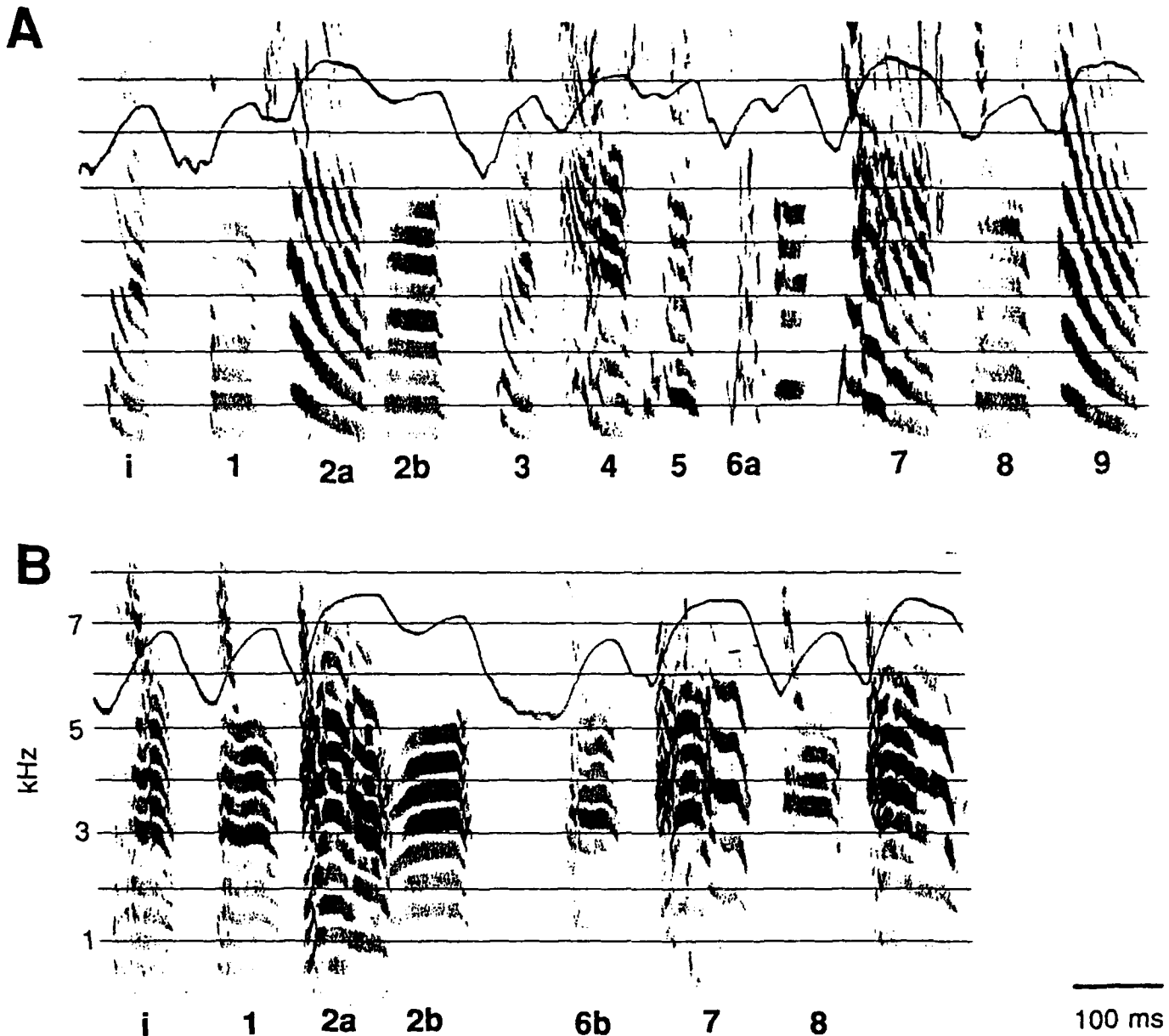
Figure 3. **Song 'splicing' after damage to the vocal motor nerves.**
A. The song of an intact adult male zebra finch. The top trace is a measure of amplitude.
B. The song of the same adult male zebra finch, as sung 35 days after the right tracheosyringeal (ts) nerve was cut and 15 days after the left ts nerve was cut. Although syllable morphology has changed, the amplitude trace and the length of the syllables allows matching to the intact song. Note that syllables 3, 4, 5, and 6a have been lost and that the time between the delivery of syllables 2b and 6b has decreased from 330 ms to 100 ms.

- 13 -

"Timbre" control in zebra finch song

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For Ethology and Ecology

Millbrook, NY

89 2 15 245

Abstract

Zebra finch song syllables often include harmonically related frequency components. Some of these "harmonics" may be suppressed while others are emphasized. This differential emphasis varies between the syllables in a song and between individuals' songs. We call these patterns of harmonic suppression "timbre".

Patterns of harmonic suppression seen in individual syllables are conserved within adult males' songs for periods of at least nine months. Young males that imitate the songs of older males also imitate their patterns of harmonic suppression. Syringeal denervation grossly distorts these patterns of harmonic suppression, which suggests that they result from active control of the vocal organ.

The selective suppression and emphasis of some harmonics creates a great number of possible timbre variants for any one syllable. These add signal diversity to the limited array of frequency modulations and range of fundamental frequencies found in zebra finch song. Analyses of bird song that disregard differences in patterns of harmonic emphasis and suppression may overlook a feature that is important in vocal communication.

A vocalization, as a sound wave, can be completely characterized by reference to four variables: frequency, time, amplitude, and phase. Different methods of sound analysis tend to emphasize two or three of these parameters, with a consequent loss of information. When such methods are used in studies of vocal communication, one should weigh the importance of the information lost. In the study of animal vocalizations, the most widely used method of analysis is the sonogram (Thorpe, 1958; Marler, 1969; Bertram, 1970; Clark, Beeman, and Marler, 1987). Sonograms represent frequency as a function of time extremely well, but reduce amplitude information to a gray scale and discard phase information (Koenig, Dunn, and Lacy, 1946; Potter, Kopp, and Green, 1947; Joos, 1948). The oscillogram (e.g., Greenewalt, 1968) plots amplitude as a function of time, but frequency and phase information are obscured in sounds with more than one frequency. Zero-crossing analysis (West et al., 1979) accurately represents frequency only for sounds without harmonics.

Most researchers using these methods to describe avian vocalizations do not measure the amplitude of song components. Apart from the biases introduced by the method used in representing the sound, some additional reasons for ignoring amplitude information exist: 1) recordings are usually made so as to both maximize signal salience and avoid overloading (resulting in the recording of softer sounds with greater gain, so that all amplitudes appear equally dark on a sonogram); 2) the distance from the source is usually not specified, and amplitude comparisons may not be meaningful; 3) the orientation of the bird towards the microphone is not controlled, though it affects the amplitude of the sounds recorded. An additional confounding variable is the "Hi-shape" setting on the Kay sound spectrograph, which is used by many researchers without considering its effects: this circuit distorts the signal by decreasing its amplitude by 6 dB/octave up to 9 KHz. These various biases have tended to obscure or distort the representation of amplitudes within complex animal vocalizations. In contrast, our understanding of how information is encoded within human speech (which has been compared to song because of their many common characteristics - see Marler, 1970, Marler and Peters, 1981) relies heavily upon the description of frequency bands with different amplitudes (or formants) within a single vocalization (Fant, 1960).

Most zebra finch syllables include a very wide range of frequencies. Although the fundamental frequencies of individual syllables span a rather narrow range (between 400 and 2000 Hz), the acoustic energy in song syllables is concentrated between 500 and 6500 Hz. Most this energy occurs in peaks that are multiples of the fundamental, and so can be assumed to harmonics. Song syllables with 10-12 harmonics (Fig. 1a) within the zebra finch audibility range (500-6000 Hz; Okanoya and Dooling, 1987) are common. The possibility that the relative amplitude of the harmonics may constitute an important signal parameter has been overlooked by previous investigators. We refer to the characteristic differences in the amplitudes of a syllable's harmonics as its "timbre". Evidence presented here argues that timbre is syllable-specific, learned by imitation, and the result of active vocal control.

Method

Subjects

Zebra finches *(Poephila guttata)* are a small (10 g) colonial Australian finch. Young males learn their songs from adults during an early sensitive period; after sexual maturity is reached at 90 days the song is fixed (Immelmann, 1969; Price, 1979). Zebra finches sing and maintain pair bonds throughout the year (Immelmann, 1965); breeding is unpredictable and initiated by rainfall(Farner and Serventy, 1960). Courtship and song are performed year-round at close range within the colony (Immelmann, 1965).

We used 44 adult male zebra finches. Most of these birds were bred in our own colony. Sixteen of the birds learned their songs from live conspecific tutors that had been previously recorded.

Procedure

Sound recordings were made with a Tandberg reel-to-reel or a Marantz portable cassette recorder. Birds were recorded while singing "directed" song (Sossinka and Böhner, 1980). Sonograms were made with a Kay Digital Sonagraph, using the 300 Hz filter ("wide-band"), recording from 0-8000 Hz without the use of the Hi-shape filter.

The sonograms were examined and 12 songs were chosen that a) did not share syllables with the other songs and b) had at least four syllables suitable for Fast Fourier Transform (FFT) power spectrum analysis (i.e., syllables which contained sections lasting at least 12.8 ms without frequency modulation). These songs were low-pass filtered (10 KHz) and then digitized at 20 KHz using a Data Translation 2801A board in an IBM PC-AT computer. FFT-based power spectra of the syllables or syllable sections of interest were computed and plotted using ASYST software, and the frequencies and amplitudes of spectral peaks determined by placing a cursor on the peak and printing out the coordinates (algorithms and code for these procedures are available upon request). The amplitude values, expressed in dB,   were then normalized so that they could be directly compared: the value for each spectral peak was expressed as the difference between that peak's value and

the peak value of the loudest harmonic in that syllable. The syllable's highest-amplitude harmonic was thus assigned an amplitude of 0, with other spectral peaks receiving values such as -3.5, -7.0, and -29.5 dB, denoting their difference in amplitude from the loudest peak.

Young zebra finches normally acquire their songs by imitating the songs of older conspecifics (Immelmann, 1969. The songs of tutors and imitators with at least four syllables suitable for spectral analysis were digitized and analyzed.

Four adult male zebra finches were re-recorded 9 months after initial recordings were made.

The tracheosyringeal nerve innervates the muscles of the trachea and of the avian vocal organ, the syrinx, and is necessary for the production of normal song (Nottebohm et al., 1976). Nerve sections were performed upon four adult zebra finch males to determine the effect of syringeal denervation upon the differential suppression of frequency components. For this operation, zebra finches were first anesthetized with either metofane (inhalation) or ketamine/xylazine (injection). An incision was made in the skin overlying the trachea, and the tracheosyringeal nerves were dissected free and cut just before they entered the interclavicular air sac. A portion of the nerve distal to the cut was removed to inhibit regrowth. After a one-month recovery period, the opreated birds were recorded again, and then both nerves were visually inspected to determine the extent of regrowth, if any. Only animals without apparent nerve regrowth were used. Thier songs as recorded before and after nerve section were digitized and analyzed as described above.

Results

1. Fundamental frequencies and harmonics in zebra finch song syllables.

The fundamental frequencies of the 69 notes analyzed in this study ranged from a minimum of 410 Hz to a maximum of 2030 Hz. However, the vast majority of syllables (74%) had fundamental frequencies between 500 and 700 Hz.

The vast majority (63 of 69, or 91%) of zebra finch syllables analyzed consist of a fundamental frequency and a series oι components that are multiples of the fundamental.   We call these multiples of the fundamental "harmonics". In some cases (e.g. syllable 3b in LG96's song) one or more frequency components are unevenly spaced, and so the syllable cannot be considered a simple harmonic series. When analyzing amplitude as a function of harmonic order, such syllables are not considered (except in one case: LG96 and LBY; Fig. 7).

2. Individual syllables within one bird's song differ markedly in relative amplitude of harmonics.

Amplitude differences in the harmonics of single song syllable are sometimes large enough to be seen by simple visual inspection of sonagrams. Syllable 6b in the song of zebra finch male Y45 (Figure 1a) is an example of such a syllable. Five frequency components can be seen clearly in this sonagram; the upper four are spaced evenly at 650 Hz intervals, and the lower component has a frequency of 1300 Hz, which would be the 2nd harmonic of a series with a 650 Hz fundamental. The expected first (650 Hz), third (1950 Hz), and 8th and higher harmonics cannot be seen in this sonogram. When the FFT power spectrum of this syllable is examined (Fig. 1b), we see that the first harmonic, or fundamental, indeed exists, but is depressed by more

than 40 dB relative to the 2nd harmonic. Likewise, the third harmonic is suppressed by 16 dB relative to the 2nd harmonic and 11 dB relative to the 4th harmonic. This syllable, then, has two regions of frequency emphasis (the 2nd harmonic at 1.3 KHz and the 4th-7th harmonics at 2.6-4.55 KHz) and three regions of suppression (the 1st harmonic, at 650 Hz, the 3rd harmonic, at 1.95 KHz, and the 8th and higher harmonics, above 5 KHz).

The spectra of six other syllables (or portions of syllables suitable for FFT analysis - see Procedure) from the song of Y45 have widely differing patterns of harmonic suppression (Figs. 1a and 1b). For example, compare the spectra drawn from syllables 4 and 5. The fundamental frequencies of the two syllable segments that were analyzed differ only by 5 Hz (550 and 555 Hz), yet their harmonics' amplitudes differ dramatically. When the relative amplitudes of the harmonics in the two syllables are plotted against each other (Fig. 2), it can be seen that there is no significant relationship between harmonic order and relative amplitude ($r = .264$). Since syllables 4 and 5 have nearly identical frequencies, the low correlation coefficient also implies that there is no significant relation between the frequency of a harmonic and its amplitude. Correlation coefficients were determined for all possible pairs of the seven analyzed syllables in Y45's song; the average r for the 21 comparisons was .402, with a range of -.265 (syllables 1 and 4) to .858 (syllables 5 and 6).

The normalized amplitudes of each spectral peak for each harmonic in the seven syllables from Y45's song were plotted as a function of frequency (Fig. 3a) and harmonic order (Fig. 3b). A noticeable decrease in peak amplitudes can be seen above 4500 Hz (or the 9th harmonic), and maximal peak values (those with a relative amplitude near 0 dB) tend to fall between 1000 and 4500 Hz (2nd through 8th harmonics). However, harmonic suppression occurred at all frequencies. The range of relative suppression varies from 10 dB (-2 to -12 dB, 2000 Hz, 3rd harmonic) to 34 dB (-7 to -41 dB, 500 Hz, 1st harmonic). Thus the relative amplitude of each harmonic within the syllables in this bird's repertoire can be varied over at least a three-fold (i.e. 10 dB) and as much as a fifty-fold (i.e. 34 dB) range.

3. Consistency in the relative amplitude of harmonics across time.

The patterns of harmonic suppression seen in an individual zebra finch male's song syllables were similar or identical to the timbre of other examples of corresponding song syllables drawn from a) songs delivered during the same recording session and b) songs of the same male recorded 9 months later.   Sonograms of a typical example of such consistency in the patterns of suppression of frequency components of a syllable are shown in Figure 4. Syllables 4 and 5 in Bk89's song are suitable for FFT power spectrum analysis, and the correlation coefficients denoting the similarity of the patterns of harmonic suppression are shown for these two syllables. A total of 4 examples of syllable 4 (2 from each recording session) and 5 examples of syllable 5 (3 from the first session and 2 drawn from songs recorded 9 months later) were analyzed. The average correlation between the amplitudes of harmonics in all possible pairs of syllable 5 was very high (avg. $r = .847$; range = .737 to .996), and the average correlation coefficient for all pairwise comparisons of syllable 4 was even higher (avg. $r = .870$; range = .817 to .987). The similarity of syllable timbre for pairs of homologous syllables drawn from the same recording session (avg. $r = .928$) was higher than that of syllables drawn from recording sessions separated by 9 months (avg. $r = .813$); but it should be noted that all correlation coefficients for pairs of homologous syllables exceeded significance ($p < 0.01$).

It is possible that the lower harmonics, with a larger representation within the auditory system (as shown by critical ratios - see Okanoya and Dooling, 1987) might be more prominent to the zebra finch and show less (or more) variability in their patterns of harmonic suppression. However, consideration of only the first four harmonics in comparing homologous syllables delivered within one recording session gave a correlation coefficient of .908, while using the first 10 harmonics in the comparison yielded an $r = .927$. The stereotypy and accurate replications of the patterns of harmonic suppression extend uniformly over the entire audibility range of the zebra finch.

4. Suppression of harmonics in syllables drawn from within a population of zebra finch songs.

Relative amplitudes of frequency components from 69 syllables, drawn from the songs of 12 zebra finch

males with differing songs, were normalized and plotted as a function of frequency (Fig. 5a) and harmonic order

(Fig. 5b). The population trends seen here in the analysis of harmonic suppression and emphasis are

consistent with those found within one individual's song, as described above. The greater resolution resulting

from the increase in number of syllables allows us to define three frequency regions (500-800 Hz, 1st

harmonic; 1700-2200 Hz, 3rd harmonic; >5000 Hz, 9th and higher harmonics) which appear to be more prone

to suppression than other frequencies or harmonics within the audibility range (see Table 1). Although

suppression is most likely in the region of the 1st and 3rd harmonics (and least likely for the 6th harmonic), it

occurs throughout the audibility range of zebra finch song: every frequency and harmonic shows instances of

suppression by at least 33 dB (Fig. 5).

One class of syllable that is of particular interest in the context of this study is the "high note" often seen

between 3 and 6.5 KHz in zebra finch songs. This syllable appears in sonagrams as a single, high-frequency

element (Fig. 6a, syllable 5). Examination of the power spectra of such notes (Fig. 6b) reveals that such

syllables are extreme examples of the harmonic suppression which is seen to some degree in nearly all

syllables.

The locations of regions of harmonic suppression within individual syllables were tabulated by harmonic

order. All harmonics or series of adjacent harmonics with amplitude peaks at least 7.5 dB below the average of

the two higher-amplitude components delimiting that series were considered regions of suppressed amplitude

(see Cynx, Williams and Nottebohm, submitted, Fig. 3). The number of occurrences for each observed pattern

of harmonic suppression within the 69 syllables is shown in Table 2. A total of 29 different patterns of

frequency suppression within a syllable were seen. The four most commonly observed patterns (including

syllables without any suppressed harmonics) account for 46%, or nearly half, of the syllables analyzed, and 29

of the syllables, or 42%, have patterns of suppression that correspond to some combination of the 1st, 3rd, and 4th harmonics. However, 46% of the syllables had patterns of harmonic suppression that could not be predicted from the general trends of frequency and harmonic suppression (as shown in Fig. 5). No strong tendency for patterns consisting of only odd or even harmonics was observed: only 6 of 22 patterns (27%) and 13 of 38 syllables (34%) with two or more suppressed harmonics were composed exclusively of odd or even harmonics. Most patterns (17 of 29, or 59%) of harmonic suppression were observed only once.

A total of 99 different regions of suppressed amplitude and 139 suppressed harmonics were found in the 69 syllables analyzed. The third harmonic is the most commonly suppressed, followed by the first, fourth, and fifth harmonics; two or more examples of suppression were found for each harmonic except the ninth (Table 3). The number of discrete regions of suppressed harmonics within the audibility range was determined for each of the 69 syllables: 9 syllables (13%) had no suppressed harmonics, 29 syllables (42%) had one region of harmonic suppression, 23 syllables (33%) had two separate regions of harmonic suppression, and 8 syllables (12%) had three regions of harmonic suppression.

The observed variations in patterns of harmonic suppression might correspond to a function of fundamental frequency. To examine this possibility, ten syllables from among the 69 analyzed were selected on the basis of length, frequency modulation, and fundamental frequency. All were virtually unmodulated, at least 45 ms long, and fell into one of three narrow ranges of fundamental frequency (512-519 Hz, 577-585 Hz, 645-657 Hz). No two syllables in a frequency class were drawn from the same bird. Table 4 shows the correlation coefficients (of amplitude by harmonic order) of comparisons between pairs of syllables within a frequency class. The average correlation for the 12 pairs of syllables with similar frequencies is not significant ($r = .299$) and every comparison (r range $= -.293$ to $.631$) falls above the 0.05 level of confidence.

The spectral peaks which correspond to the harmonics emerge from noise, and the noise level varies with frequency (see the power spectra shown in Figs. 1b and 6b). Table 5 shows that syllables with similar frequencies are more alike when one compares the amplitude difference between peak and noise than when peaks alone are considered. However, the relationship between frequency and the peak-to-noise amplitude

difference does not reach significance and can account for at most 25.6% of the variability seen in the patterns of harmonic emphasis and suppression.

5. Patterns of harmonic suppression are learned.

Young male zebra finches alter the sequence and morphology of the syllables they sing so as to match a model (Böhner, 1983; Williams, submitted). In addition, the amplitudes of harmonics within a copied syllable appear are often reproduced with remarkable fidelity. Figure 7 shows an example of this correspondence of syllable timbre in the tutors' and pupils' songs. In this case, the young zebra finch, LG96, was determined to have copied portions of his song from two different adult males, LBY and RW, on the basis of syllable morphology and sequence, and without consideration of timbre (Williams, submitted). RW was the male in attendance at the nest where LG96 was hatched, and thus was the presumed father. LBY and RW were known to be unrelated for at least three generations; the similarity measure for their songs was .09 on a scale of 0 to 1 (see Williams, submitted).

As can be seen by comparing sonograms (Figure 7), the latter portion of LG96's song (syllables 4a-8b) was copied from adult male LBY (syllables 3b-7); the source for this song could only have been LBY as no other potential song tutor sang a similar song. When the patterns of harmonic suppression for all the syllables in LG96's song and syllables 3b-7 in LBY's song are compared, the correlation coefficients cover a range between -.214 and .949, with the average r = .213. However, when only the four syllables which match by sequence and are suitable for spectral analysis within the two songs (3b and 4b, 4b and 5b, 5 and 6, and 8 and 9 - see Fig. 7) are compared, the average r = .868 (range = .745 to .949), a highly significant correlation.

The initial portions of three additional adult male zebra finches' songs were similar to RW's song and could have provided the model for the initial portion of LG96's song (syllables 1-3b). All four of the potential models (RW, DB, LB, LG) had four syllables with morphology corresponding to the four initial syllables of LG96's song.

Three of the four models (DB, LG, and LG) had additional syllables interposed between the syllables corresponding to LG96's syllables 2 and 3a (see Fig. 7). In the song of the fourth adult model, RW, the first four syllables corresponded in sequence as well as morphology to the first four syllables of LG96's song. When the patterns of harmonic suppression for portions of the first two syllables in each of the five songs (LG96 and the four models) were compared, all syllable pairs were found to have relatively high correlation coefficients (avg. $r =$ .409 for syllable 1, avg. $r =$ .545 for syllable 2). The correlation coefficients for the comparisons between LG96's syllables 1 and 2 and each of the four models were then averaged: RW, with an average $r = .684$, provided the only instance of a model with significant correlation to LG96's first two syllables. RW is also the putative father of LG96, and the sequence of RW's song most closely matches the sequence of the first portion of LG96's song. These factors suggest that the pattern of harmonic suppression was copied along with the model's syllable morphology and sequence.

The patterns of harmonic suppression in the two portions of the song that were copied from different models show little similarity. When the patterns of harmonic suppression in syllables 1 and 2 of LG96's song were compared to those in syllables 4, 5, 6, and 8 (also from LG96's song, but copied from LBY) the average correlation coefficient was .075 (-.214 < r < .55), indicating that the patterns of harmonic suppression in each portion of the song (and in each model song) are unrelated.

It is interesting to note that LG96's syllable 3b, which ends the portion of the song copied from RW, seems to have hybrid properties and act as a bridge between the two parts of the song. By position and morphology this syllable corresponds to syllable 3b in RW's song, and the correlation coefficient for timbre of these two syllables is high ($r = .668$). Yet the pattern of harmonic suppression in LG96's syllable 3b is even more similar to that seen in syllable 3b from LBY's song ($r = .838$). Recall that syllable 3b from LBY's song corresponds to and provided the model for the morphology, sequence, and timbre of LG96's syllable 4b. Close examination of LG96's syllable 3b (see Fig. 7), and the corresponding syllables from the two song models (LBY's 3b and RW's 3b), reveals that while frequency components 4, 5, and 6 of RW's song are evenly spaced harmonics, the same components in LG96's and LBY's syllables are unevenly spaced non-harmonic

frequencies.   Although the position and length of LG96's syllable 3b match those of the RW song model, the

syllable's frequency structure and timbre more closely resemble those in LBY's song. It seems possible that

LG96's syllable 3b is an improvised amalgam, in that it combines characteristics from LBY's syllable 3b and RW's

syllable 3b to form a bridge between the two portions of the song which were learned from different models.

6. Neural control of the harmonic suppression.

Figure 8 shows sonograms of ten syllables from Y45's and DG50's songs which could be identified after

tracheosyringeal nerve section by their position and duration, along with the versions produced by the intact

bird. The average fundamental frequency of syllables delivered after nerve section was 141 Hz lower than the

same syllables sung by the intact bird. Gross changes in patterns of harmonic suppression also appeared after

nerve section.

FFT-based power spectra were generated for the twenty syllables, and correlation coefficients for the

comparisons between the patterns of harmonic suppression for each syllable as delivered before and after 's

nerve section were determined. The relative lack of similarity between the timbre of syllables delivered before

and after syringeal denervation is confirmed by this analysis (avg. $r = .378$ for the 10 syllable comparisons; recall

that syllables delivered by the same bird at 9-month intervals all had $r > .800$). Inter-syllabic similarity in patterns

of harmonic suppression within an individual's song was relatively higher after nerve section (avg. $r = .681$) than

in the intact birds' songs (avg. $r = .376$). This trend may indicate that patterns of harmonic suppression within a

song become more homogeneous when the vocal organ is denervated.

The normalized amplitudes of harmonics from the ten syllables as delivered before and after nerve

section are plotted as a function of frequency in Figure 9a (amplitude was not plotted as a function of harmonic

order because of the consistent difference in frequency after nerve section). The distribution of peak values of

harmonics produced by nerve-sectioned birds is compressed in range and nearer to the maximal peak value

throughout most of the audible frequency range. The most marked difference between the two distributions, however, is the fequency region below 2500 Hz, where the maximal amplitudes of harmonics delivered after nerve section is markedly depressed relative to harmonics at the same frequencies in intact birds' syllables. At 1000 Hz, there is a 10 dB difference between the highest peak amplitudes from the intact and nerve sectioned birds' songs. This relative emphasis of harmonics above 2500 Hz and suppression of harmonics below 2500 Hz in the nerve-sectioned birds' songs is also evident in the sonograms (Fig. 8).

Relative peak-to-noise levels were determined for each harmonic by subtracting the noise level at the frequency of a spectral peak (see spectra in Figs. 1b and 6b) from the peak's amplitude. This measure of peak amplitude, when normalized with respect to the highest peak-to-noise level within a syllable and plotted as a function of frequency, also distinguishes between the patterns of harmonic suppression found in the syllables of intact and nerve-sectioned birds (Fig. 9b). The signal-to-noise values of harmonics in syllables sung after ts nerve section have more uniform amplitudes (i.e., are clustered closer to 0 for all frequencies), and do not show the extremes of harmonic suppression (i.e., fall within a narrower amplitude range) seen in syllables drawn from the intact birds' songs. Harmonic amplitudes within the syllables of ts nerve-sectioned birds can then be said to fall at a relatively constant level above the spectral noise level, while the harmonic amplitudes in syllables sung by intact zebra finches vary dramatically in how far they emerge from the noise.

Discussion

The zebra finch, a colonial nester, directs its song to conspecifics at extremely close range - most often less than 25 cm (Immelmann, 1965, 1969). At this distance, there is little selective attenuation of frequencies due to environmental factors affecting trnasmission. The patterns of harmonic suppression in the present study would not be degraded and would be available to the hearer.

The diversity of harmonic suppression patterns or timbres in zebra finch song syllables may only be restricted by the mathematical limit of possible combinations of emphasis and suppression. Since apprroximately 10 harmonics usually fall within the audibility range of the zebra finch, there could potentially be 1,023 discrete patterns of harmonic suppression. However, the amplitude of certain frequency ranges and harmonics shows a tendency to be elevated or depressed with respect to others within the song, and so some patterns of harmonic suppression are more likely than others. These limitations do not seem to be severely restrictive: in a sample of 69 syllables, which can only be called small with regard to potential variability within the species, a total of 29 different patterns of harmonic suppression were observed. This diversity is found both within a bird's song (see Y45, Fig. 1) and among a population of songs.

The potential diversity of patterns of harmonic suppression might also be decreased if a relatively fixed set of acoustic filters acted upon each syllable, and the different patterns of harmonic suppression resulted simply from variation in fundamental frequency. However, patterns of harmonic suppression were dissimilar when syllables of nearly identical fundamental frequency were compared (either within a bird's song or across birds). Comparable frequencies within the entire 500-6000 Hz range sampled varied in amplitude over a range of at least 25.

Two mechanisms can be postulated as the means for producing this diversity of patterns of harmonic suppression (Greenewalt, 1968): 1) vocal tract filtering, as occurs in human speech, could be an important means of producing differential emphasis of some frequencies in bird song; 2) source filtering, in which the

physical properties of the vocal organ differentially affect the amplitudes of the various frequencies that are produced.

In human speech, broad band noise is acted upon by a set of vocal tract filters to produce the formants, or regions of harmonic emphasis; variation in these bands is the acoustic substrate for the information encoded in speech (Fant, 1960). Avian examples of vocal-tract filtering have been described. In the oilbird, the two vocal organs are positioned asymmetrically along the bronchi (Suthers and Hector, 1985), and the resulting difference in tube lengths affects the filter characteristics of the bird's airways - yielding vocalizations with emphasized frequency bands corresponding to the lengths of the airway tubes (Suthers and Hector, in preparation). Oilbirds, however, are far larger than any songbird species, and belong to a primitive family which is not believed to learn vocalizations (Kroodsma, 1981); the frequency band which is emphasized is presumably determined genetically, and cannot be varied. In a study of the songs of birds vocalizing in a helium atmosphere, Hersch (1966) reported some changes in amplitude distribution and attributed them to vocal filtering. Similarly, Nowicki (1987) demonstrated that harmonically simple passerine songs suppress frequencies above and below those which characterize the song; with a variable bandpass filter, and speculated that changes in the vocal tract (e.g., flaring the beak) might account for changes in the center frequency of the filter. Such vocal tract filtering might account for the observed variations in patterns of harmonic suppression in zebra finch song syllables if the vocal tract could be considered as at least four relatively independent variable filters (some syllables sampled had at least four regions of harmonic emphasis). It is possible that several possible vocal tract filters may in fact exist (e.g., the trachea between the syrinx and the larynx, the pharynx, the oral cavity, and the beak). However, a difficulty exists: modeling the zebra finch airway as a resonating tube with one open end (the most favorable configuration, as the resonance is 1/4 the tube length), an airway at least 16.4 cm long would be required to resonate at a frequency of 500 Hz. Zebra finches have a body length (including beak) of 7-8 cm, and the trachea is short and straight; it seems physically impossible that such filtering could affect the first few harmonics. It is possible that higher frequencies, such as 2500 Hz (which corresponds to a resonating tube 3.3 cm long), would be affected by vocal tract filtering.

Indeed, the syllables sung by the nerve-sectioned birds emphasized the harmonics in the 2500-5000 Hz range (Figs. 8 and 9a). In these animals the sound source had been denervated, and, with the source filtering removed, the effects of vocal tract filtering were unmasked.

In considering the effects of sectioning of the tracheosyringeal nerve, it is important to take into consideration that the lower few millimeters of the trachea were denervated as well as the syrinx. However, the proportion of the larynx that was denervated was small in comparison to the bird's vocal tract, and Dr. David Vicario (pers. comm.) has found that bilateral removal of intrinsic syringeal muscles affects the patterns of harmonic suppression in a manner similar to that seen after nerve section.

Since the denervated birds lacked normal timbre control, it seems likely that the patterns of harmonic suppression seen in normal zebra finch song are produced by the vocal organ. Source filtering is not thought to be important for the production of human vocalizations, except in extremely specialized situations, because of the impedance mismatch between the vocal airways and the sound source (Fant, 1960). The relative sizes and masses of the songbird vocal organ and airways may well permit source filtering. However, the physics of the avian vocal organ is not well understood, and we can only speculate upon possible mechanisms whereby the syrinx could propagate certain frequencies better than others. Differential tension on the various axes of the tympaniform membranes might set up conditions within the membrane so as to propagate certain frequencies better than others. Source filtering of avian vocalizaitons might also occur as a result of physical interactions between the two syringeal halves, which are independently innervated and controlled (Nottebohm, 1977) and yet in direct physical contact. Such interactions have been proposed to account for the presence of some frequency components in the chickadee's "dee" call (Nowicki and Capranica, 1986). Although zebra finches do not use the same mechanism as chickadees to produce harmonically related frequency components, it is conceivable that interactions between the waveforms produced by two physically coupled syringeal halves might also differentially affect the amplitudes of harmonics within a syllable.

Both source and vocal tract filtering seem to operate in the zebra finch. The emphasis of higher

frequencies and the coupling between harmonic amplitude and spectral noise level after nerve section

suggests that vocal tract filtering occurs in the syringeally denervated zebra finch. Since harmonic amplitudes

and spectral noise levels are uncoupled when the vocal organ, or sound source, is innervated, source filtering

seems likely to be responsible for timbre patterns of intact zebra finch syllables. The syrinx itself seems likely to

play an important role in the facultative control of timbre.

How much of the variability in patterns of harmonic suppression is perceptible to zebra finches is not yet

clear. A companion paper (Cynx, Williams, and Nottebohm, submitted) demonstrates that a small differences in

the amplitude of the 2nd harmonic can be discriminated. How this discriminability translates across the 10-12

frequency components that fall within the best audibility range of the zebra finch is yet to be determined. The

psychophysics of zebra finch perception are not well known, and their possible implications for timbre

discriminability are discussed in Cynx, Williams and Nottebohm (submitted). However, the evidence presented

here suggests that timbre information extending from the 1st to the 10th harmonic is important to zebra

finches: fidelity of timbre reproduction over 9 months was extended to the 10th harmonic, as was the imitation

of patterns of harmonic emphasis and suppression. It is possible that specifying the pattern of the first four

harmonics imposes a consistently predictable pattern upon higher harmonics, which would then carry only

redundant information. Examination of Table 2 reveals that patterns of harmonic suppression differing only in

the higher harmonics exist (e.g. 1 vs. 1, 10; also 1, 3 vs. 1, 3, 7). This suggests that the relative amplitudes of

higher harmonics are not directly dependent upon the patterns of suppression of the lower harmonics.

The results reported here strongly suggest that patterns of harmonic suppression are learned from song

tutors. It is unlikely that inherited physical characteristics of the vocal organ and tract control the types of

patterns of harmonic suppression within syllables that a zebra finch produces. In the example examined above

in some detail, a young male replicated two different sets of patterns of harmonic suppression drawn from the

songs of two different adult males - only one of which could be his father. The results of present study imply

that syllable timbre is learned in the same manner as are syllable sequence and morphology. Once learned, a

syllable is delivered consistently by adult males with a specfic pattern of harmonic emphasis and suppression.

This specific imitation of syllable timbre and long-term fidelity of reproduction suggest that syllables' patterns of harmonic emphasis and suppression are indeed important to zebra finches.

A salient characteristic of zebra finch song is its rich harmonic structure. Zebra finches sing a short song (0.5 - 1.5 seconds) consisting of approximately 10 syllables which are drawn from relatively few note types. Once the song is learned in the first 90 days of development, it remains fixed for life (Immelmann, 1969, Price, 1979). While the fundamental frequencies of the 69 notes analyzed in this study fell over a range of 410 - 2030 Hz, the vast majority of the syllables (74%) had fundamental frequencies between 500 and 700 Hz[1]. It is possible that patterns of harmonic suppression serve to expand the vocal repertoire of a zebra finch population, as each syllable with a given fundamental frequency, length, and modulation, can be delivered with a variety of timbres. The "high notes" produced by zebra finches at 3-7 KHz by suppressing the fundamental frequency and the lower harmonics are extreme examples of this radiation of syllable types - and are not a different note type but merely a subclass of a more general and more common class of syllables.

Zebra finch song may point to a neglected aspect of vocal learning and communication in birds, one which could be particularly important in signals used at close quarters. Formant patterns (or "timbre", in the sense used here) are also salient features of human speech, and this may well be no accident, but one of the many interesting parallels between vocal communication in humans and birds.

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[1] The syllables and syllable portions analyzed were constrained by the requirement that frequency modulation be relatively small over the portion of the sound that was analyzed. This omits short, transient syllable segments with very rapid frequency modulation, which often have fundamental frequencies at the high end of the range.

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Table 1. Levels of harmonic suppression in 69 zebra finch syllables.

Harmonic	Mean suppression (dB) ± s.d.		Range (dB)
1	13.9	11.4	50.0
2	7.8	7.8	33.5
3	13.1	8.3	50.0
4	7.9	6.4	34.5
5	7.6	7.2	30.0
6	5.7	7.1	33.5
7	9.9	8.2	40.0
8	11.1	8.6	40.0
9	13.7	8.6	31.0
10	16.6	8.9	34.0
11	17.8	6.9	23.5
12	20.2	9.9	36.5
13	19.3	8.8	23.5
14	17.0	2.8	4.0

Table 2. Distribution and patterns of suppressed harmonics.

Pattern of suppressed harmonics	No. of syllables
3	13
None	9
1, 3	5
1, 3, 4	5
1	3
1, 10	3
3, 4	3
3, 4, 5	3
1, 2	2
1, 2, 3, 4	2
3, 5	2
3, 7	2
1, 2, 3, 6, 7	1
1, 2, 5, 7	1
1, 3, 5	1
1, 3, 7	1
1, 4	1
1, 4, 5	1
1, 5, 6, 8	1
1, 5, 7	1
1, 5, 7, 8	1
2, 3	1
2, 3, 5	1
3, 4, 5, 7	1
3, 5, 7	1
4	1
4, 5	1
5	1
6	1

Table 3. Distribution of suppressed harmonics in 69 syllables.

Harmonic	Suppressions
1	29
2	8
3	42
4	18
5	16
6	3
7	9
8	2
9	0
10	3

Table 4. Correlations of syllables with similar lengths and frequencies.

Frequency range

512-519 Hz n = 3			577-585 Hz n = 3			645-657 Hz n = 4		
Δ frq.	r_{signal}	$r_{signal-noise}$	Δ frq.	r_{signal}	$r_{signal-noise}$	Δ frq.	r_{signal}	$r_{signal-noise}$
2	-.29	.34	3	.04	.56	2	.63	.43
5	.33	.83	5	.03	.50	5	.33	.13
7	.50	.67	8	.08	.48	5	.53	.67
						7	.59	.45
						7	.22	.47
						12	.63	.54
Avg. r	.18	.61		.05	.52		.49	.45
Avg. r^2	.03	.37		.002	.27		.24	.20

Total avg. r_{signal} = .299   Total avg. $r_{signal-noise}$ = .506

Total avg. r^2_{signal} = .089   Total avg. $r^2_{signal-noise}$ = .256

Figure legends

Figure 1. Patterns of harmonic suppression in an adult male zebra finch's song syllables.

A. Sonagram of Y45's song. Syllables are labeled by number; the bar beneath syllables corresponds to the segment for which a power spectrum was generated. **B.** FFT-based power spectra for the seven syllables from Y45's song which included an unmodulated segment lasting at least 12.8 ms.

Figure 2. Two syllables with similar fundamental frequencies produced by the same bird have different patterns of harmonic suppression.

The amplitudes and frequencies of 11 harmonics in syllable 4 and syllable 5 from Y45's song were determined from their power spectra (see Fig. 1b). The fundamental frequencies of syllable 4 (550 Hz) and syllable 5 (555 Hz) differed by only 5 Hz, and the syllables' morphology was similar. The values for the peaks were normalized within each syllable by setting the maximal peak = zero and expressing all other peaks as the dB level below that maximum. This figure plots the amplitude of each harmonic in syllable 5 as a function of the amplitude of the same harmonic in syllable 4. The patterns of harmonic suppression are not closely related (r = .264).

Figure 3. Amplitudes of Y45's harmonics as a function of frequency and harmonic order.

A. The normalized amplitudes of harmonics (expressed as dB below the highest-amplitude peak within the syllable) from 7 syllables in Y45's song plotted as a function of frequency. **B.** The normalized amplitudes of harmonics from 7 syllables in Y45's song plotted as a function of harmonic order.

Figure 4.   Consistency of patterns of harmonic suppression.

A. Sonogram of Bk89's song as sung in August 1986.

B. Sonogram of Bk89's song as sung in February 1987.

Correlation coefficients are shown for the comparisons between patterns of harmonic suppression in in syllables 4 and 5. These two syllables were suitable for FFT-based power spectrum analysis, and the patterns of harmonic suppression were remarkably similar after 9 months.

Figure 5.   Harmonic amplitudes in 69 syllables from the songs of 12 birds as a function of frequency and harmonic order.

A. The normalized amplitudes (expressed as dB below the highest-amplitude peak within the syllable) of each harmonic from each of the 69 syllables plotted as a function of frequency. **B.** The normalized amplitudes of the harmonics from the 69 syllables plotted as a function of harmonic order.

Figure 6.   High notes in zebra finch song are harmonic series with suppressed lower harmonics.

A. Sonogram of DG50's song with syllables are labeled by number. The bars beneath syllables 4 and 5 correspond to the segment for which a power spectrum was generated. **B.** FFT-based power spectra of syllables 4 and 5 from DG50's song. Arrows point to suppressed harmonics from the series which includes the emphasized high note(s).

Figure 7.   Imitation of patterns of harmonic suppression in a learned zebra finch song.

A. Sonograms of portions of the songs of adult male song tutors RW, DB, LB and LG.

B. A sonogram showing the song of LG96, which hatched and was raised in RW's nest box.

C. Sonogram of LBY's song. This bird was an adult male song tutor.

Bars and arrows show the correspondences of adult males' syllables to portions of LG96's song.

<u>Figure 8</u>. Patterns of harmonic suppression change after bilateral tracheosyringeal nerve section.

A. Sonograms of ten syllables (4 from Y45's song and 6 from DG50's song) as sung before tracheosyringeal nerve section.

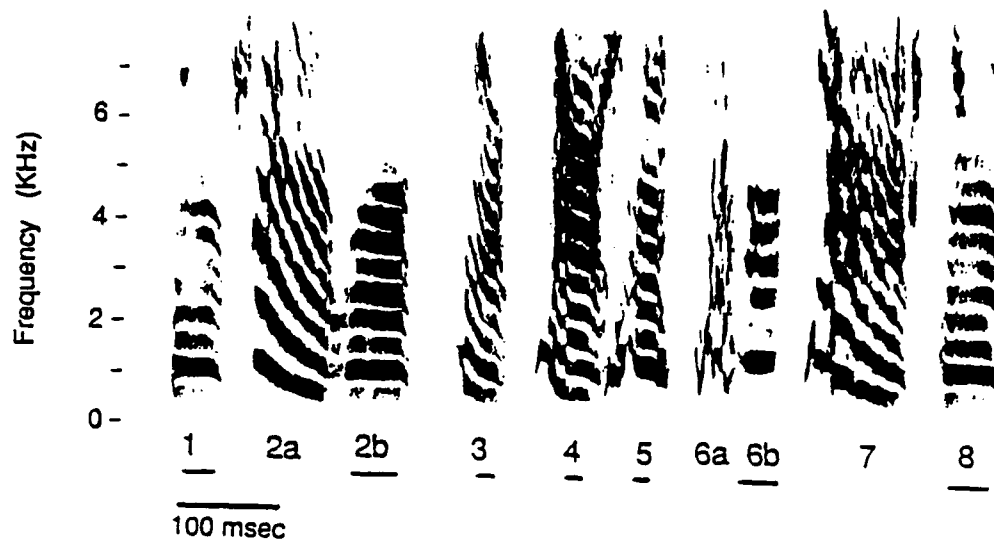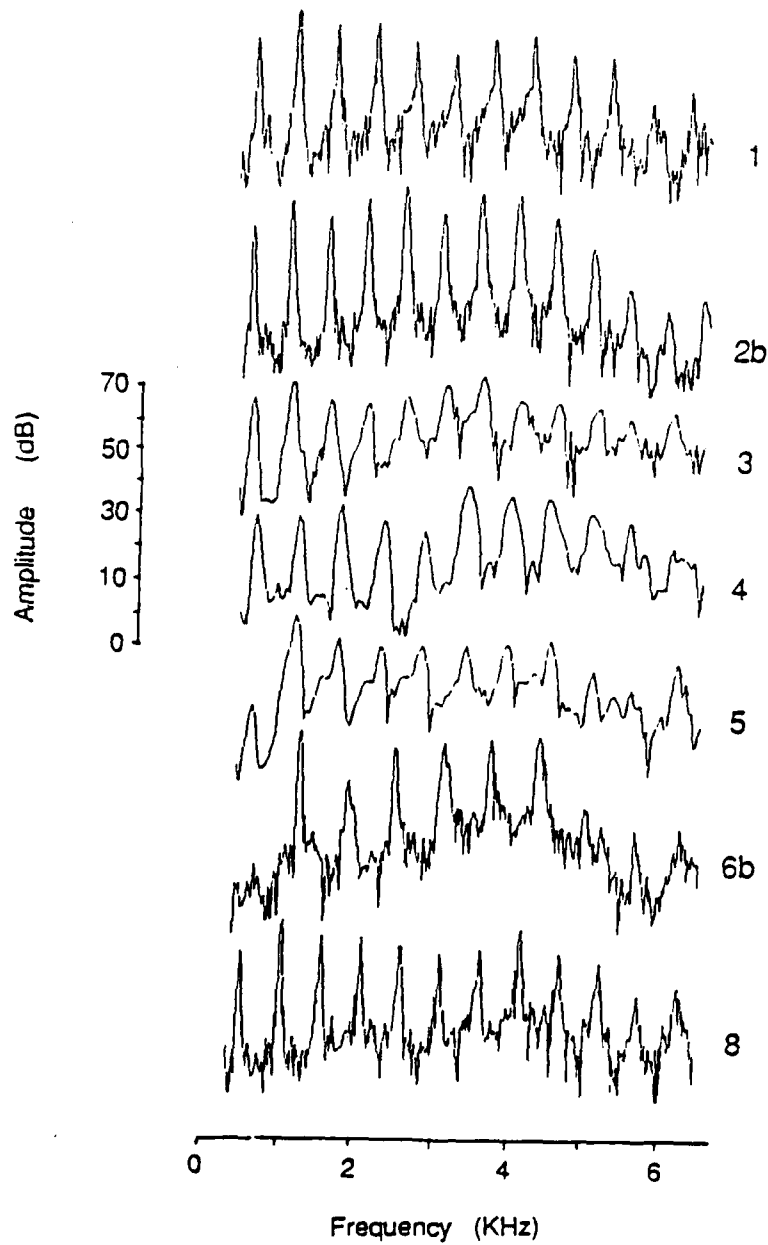B. Sonograms of the same ten syllables as delivered after bilateral nerve section.

Correlation coefficients for the comparisons between patterns of harmonic suppression as delivered before and after nerve section are shown.

<u>Figure 9</u>. Tracheosyringeal nerve section affects the range of harmonic amplitudes.

A. Normalized amplitudes (expressed as dB below the highest-amplitude peak within the syllable) of the harmonics from 10 syllables as a function of frequency. Filled circles represent harmonic amplitudes from the syllables as sung by intact birds (see Fig. 8a); open circles represent the harmonic amplitudes from the same syllables as sung after nerve section (see Fig. 8b).
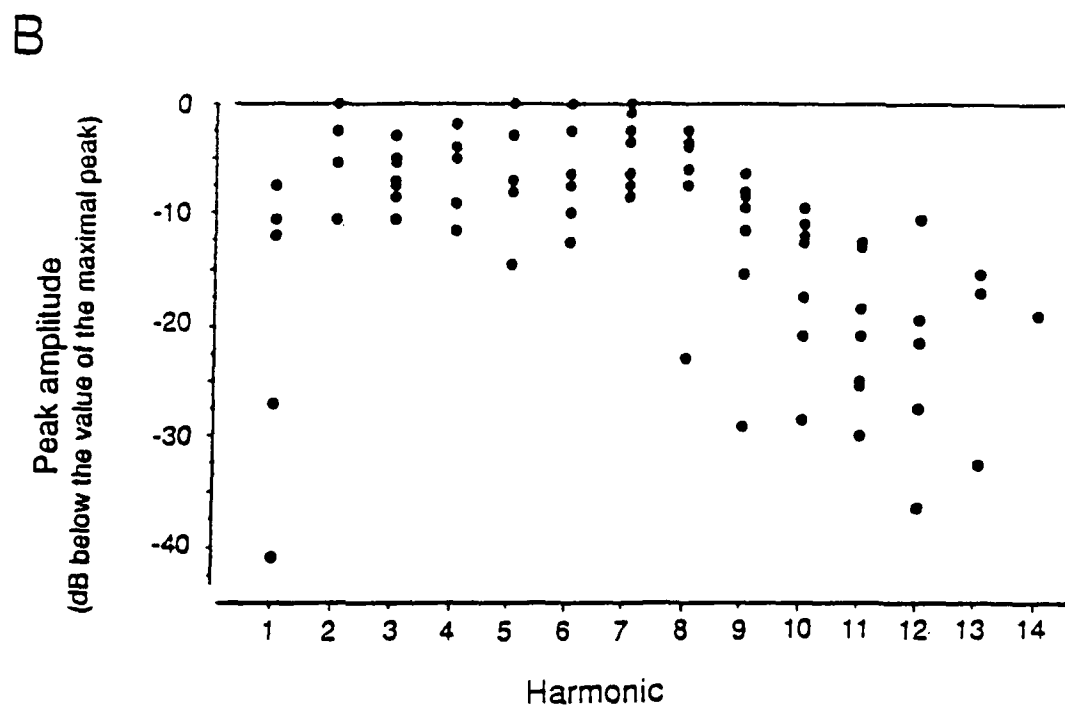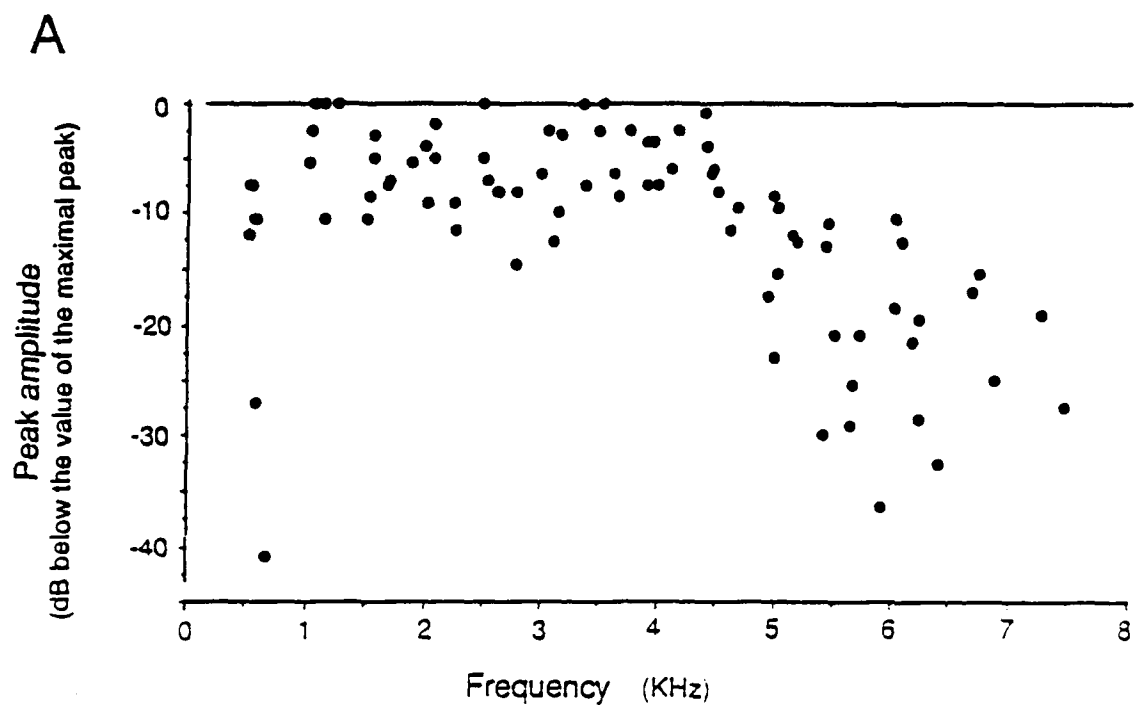
B. Signal-to-noise values were obtained by subtracting the spectral noise level from the harmonic's amplitude. The resulting signal-to-noise values were normalized as described above and plotted as a function of frequency. Filled circles represent harmonics from the syllables as sung by intact birds, and open circles the harmonics from the same syllables delivered after nerve section.
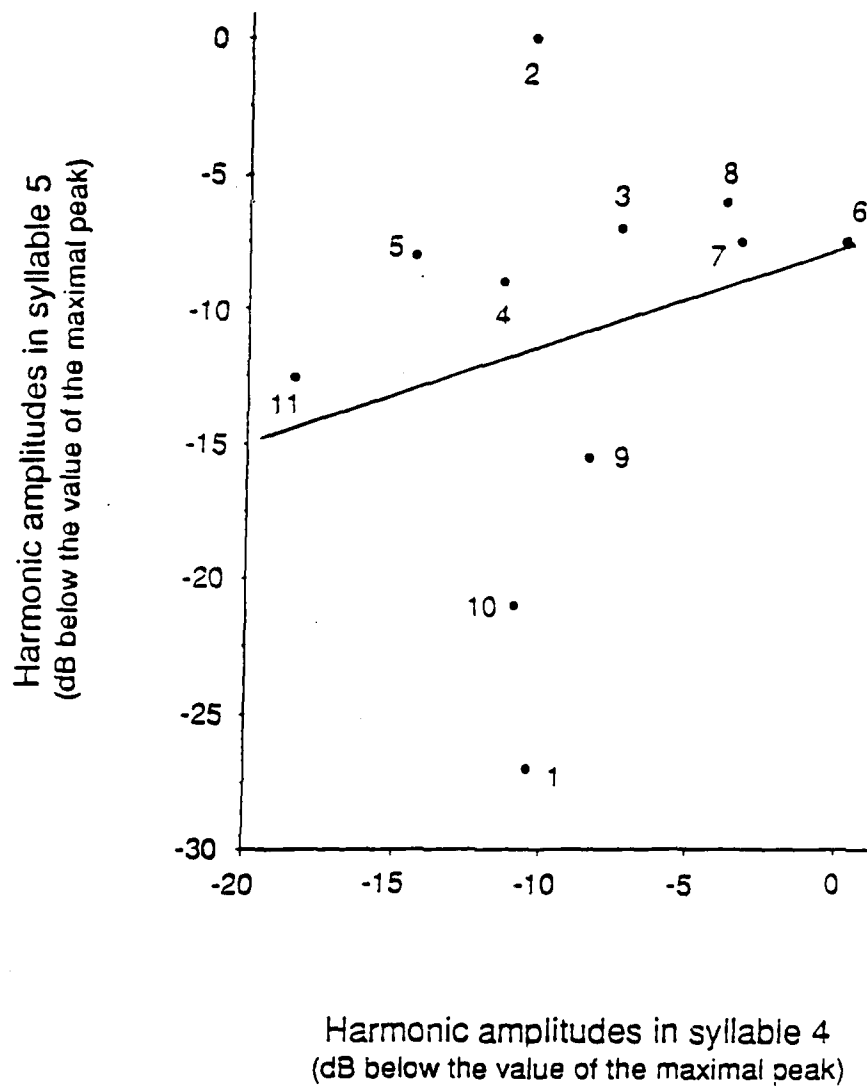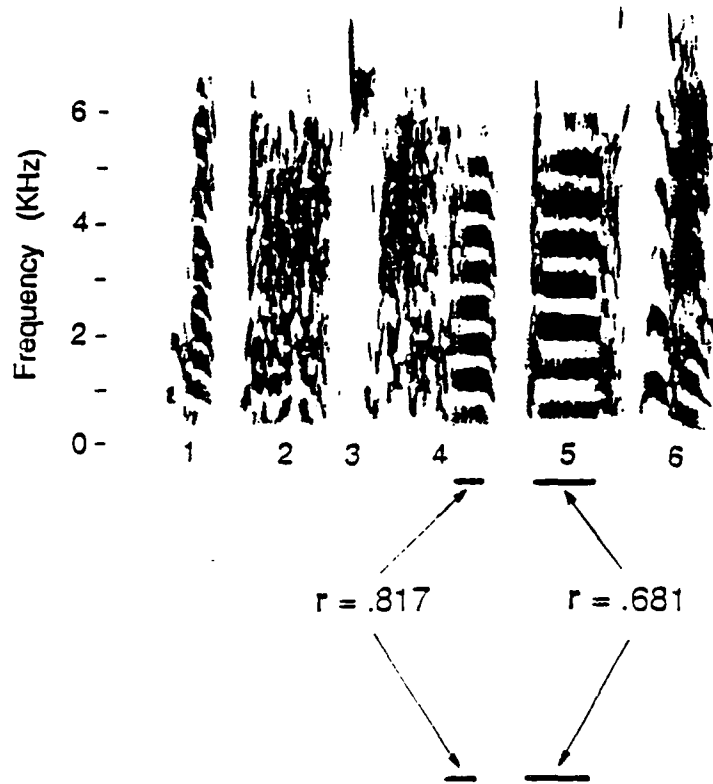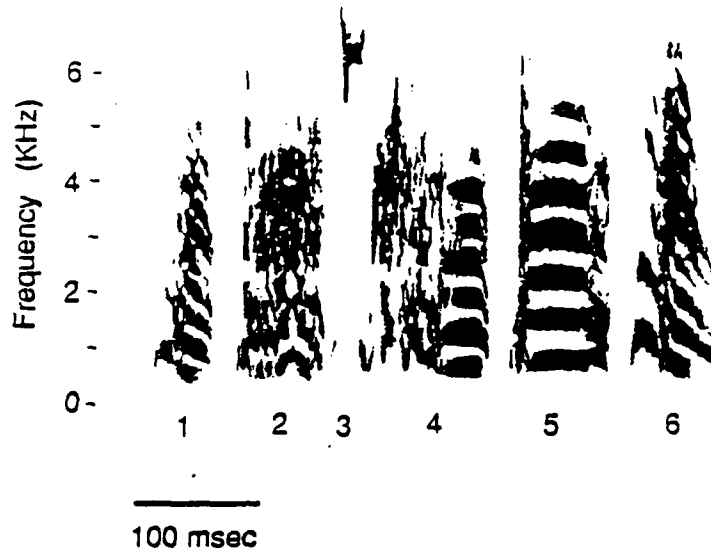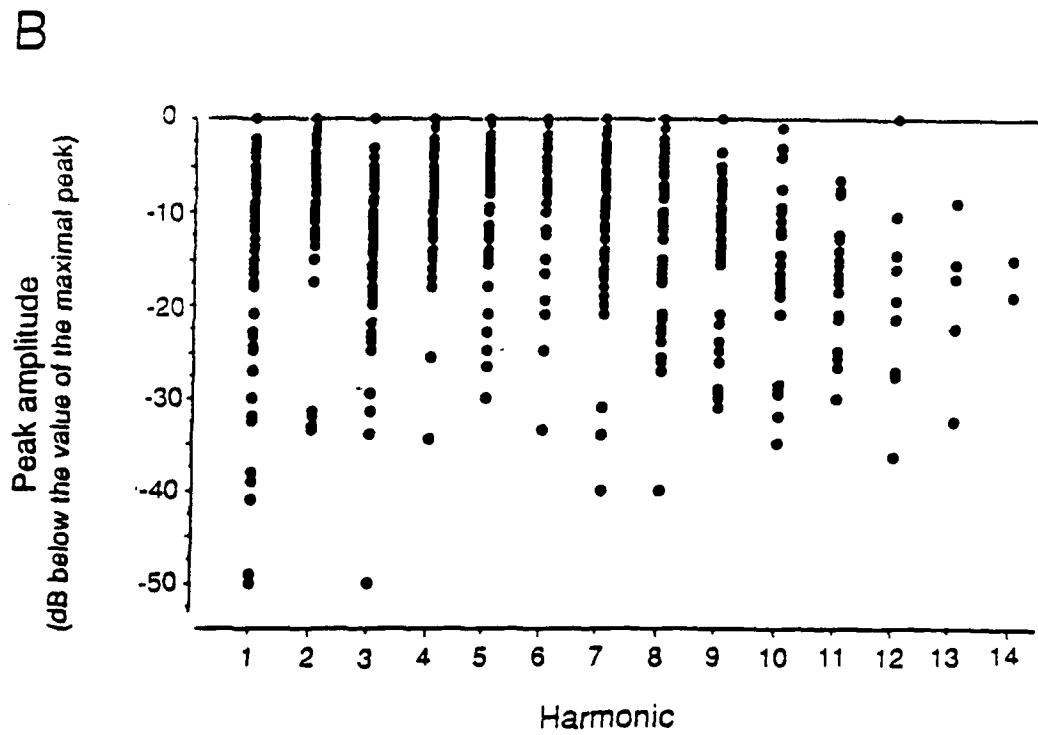
A

B

A
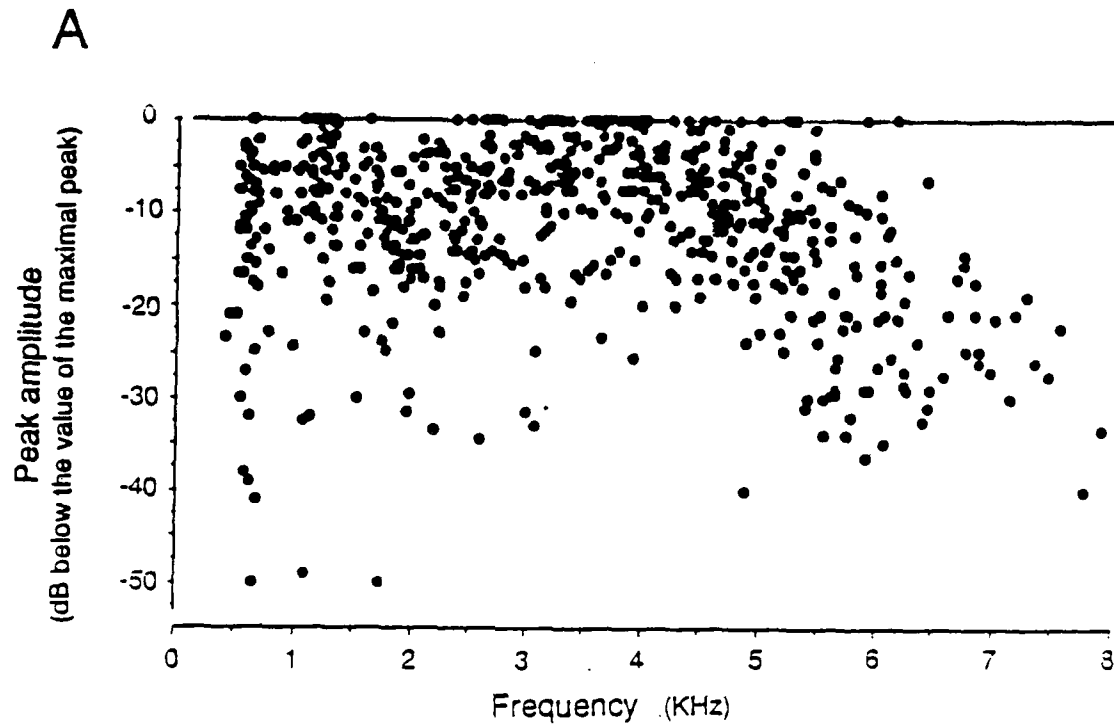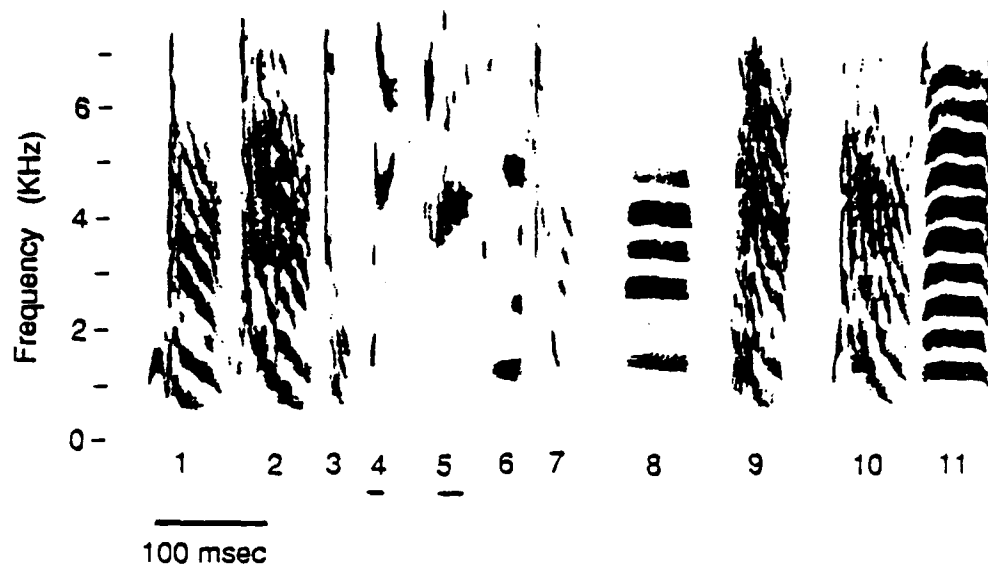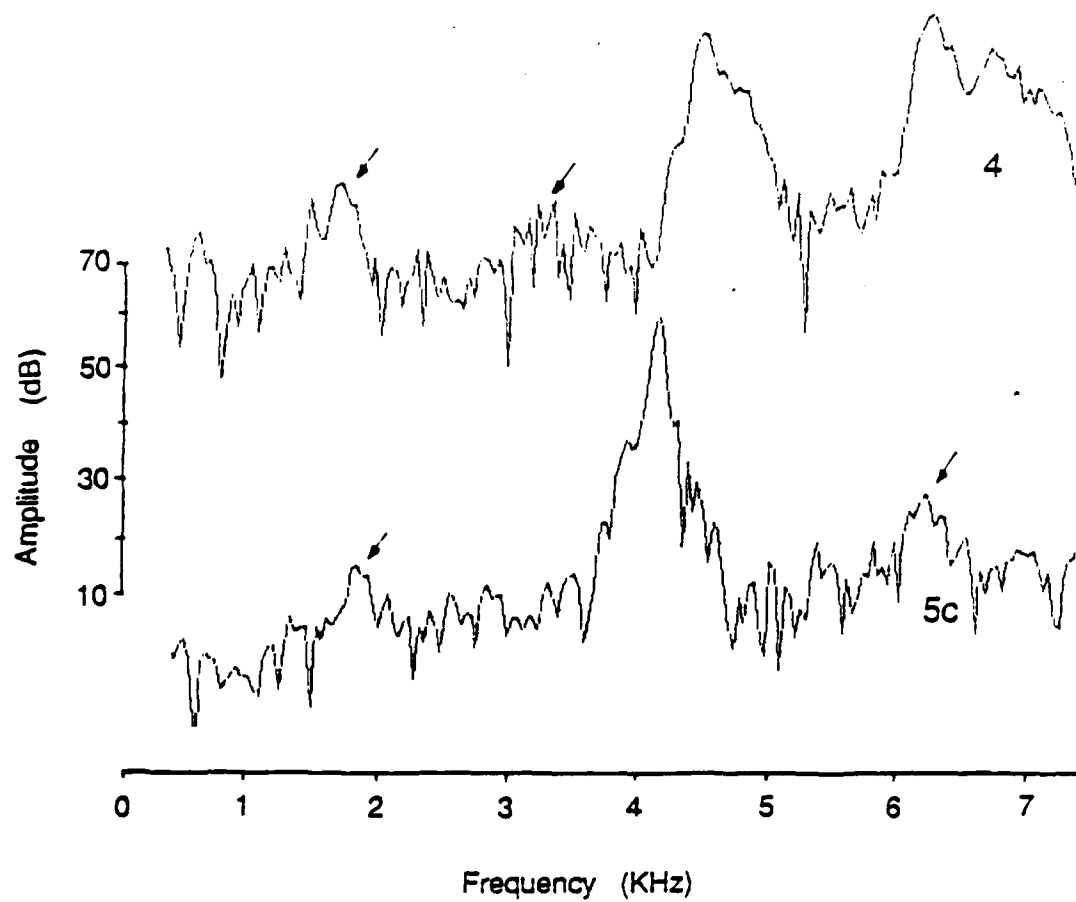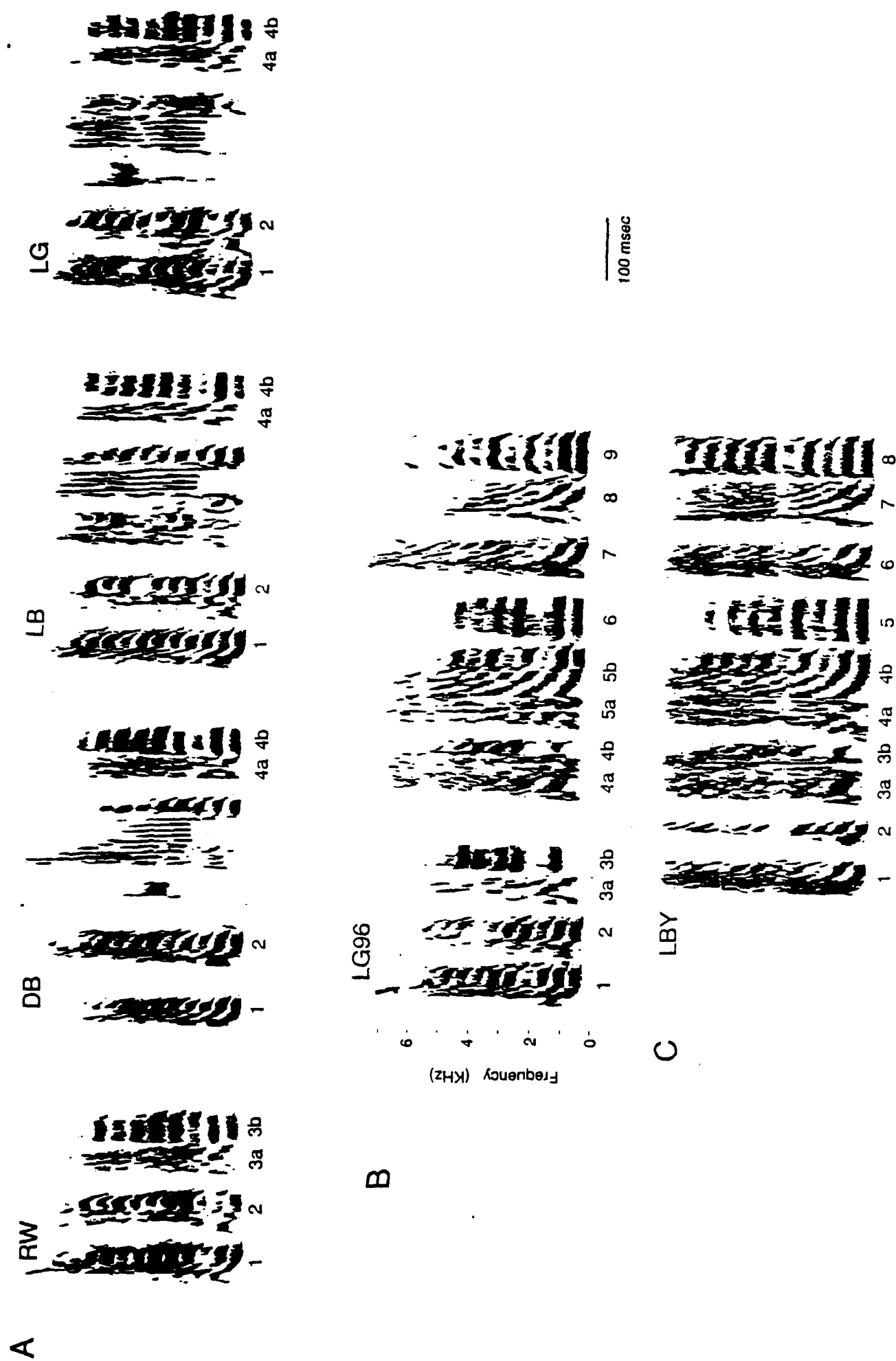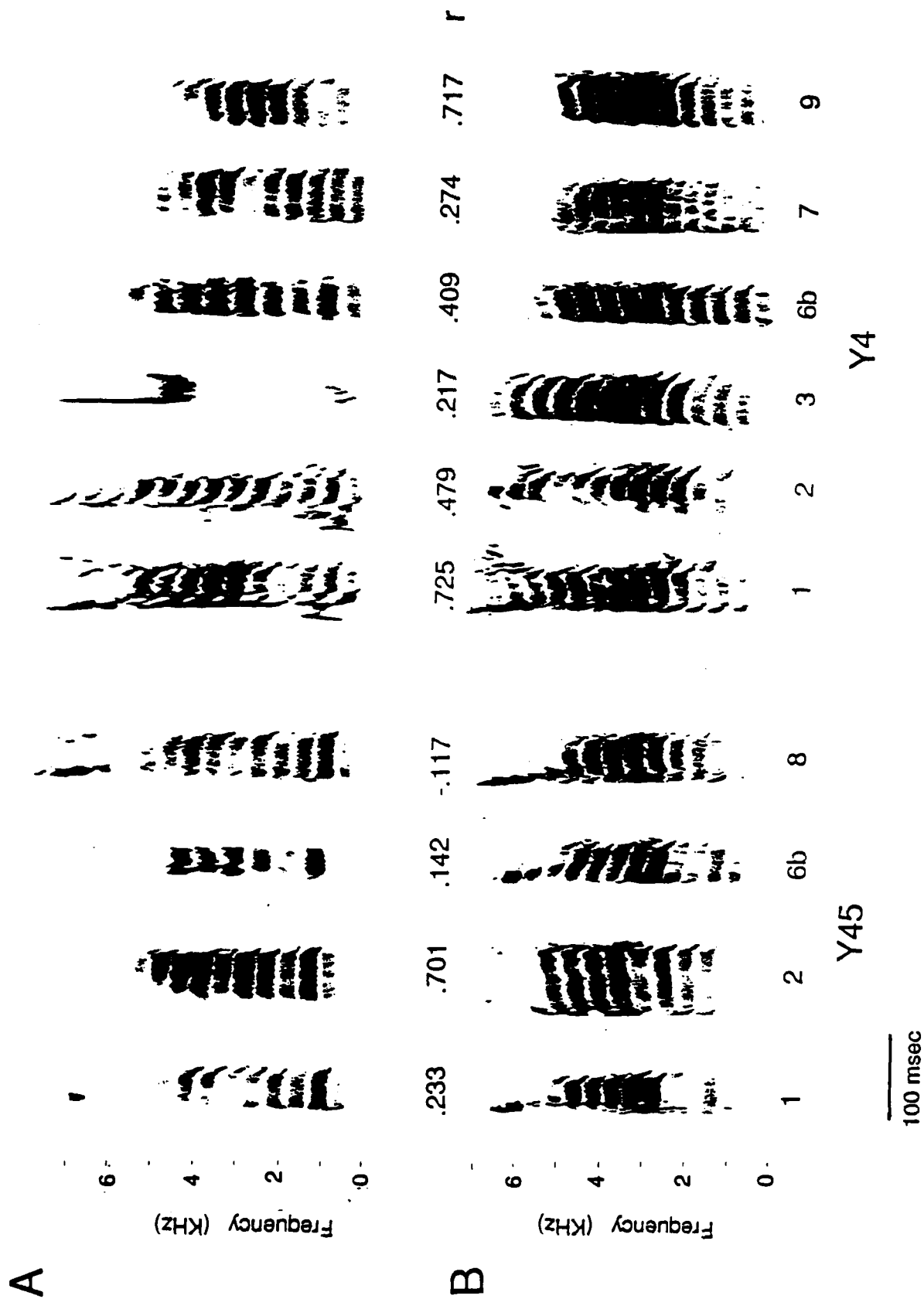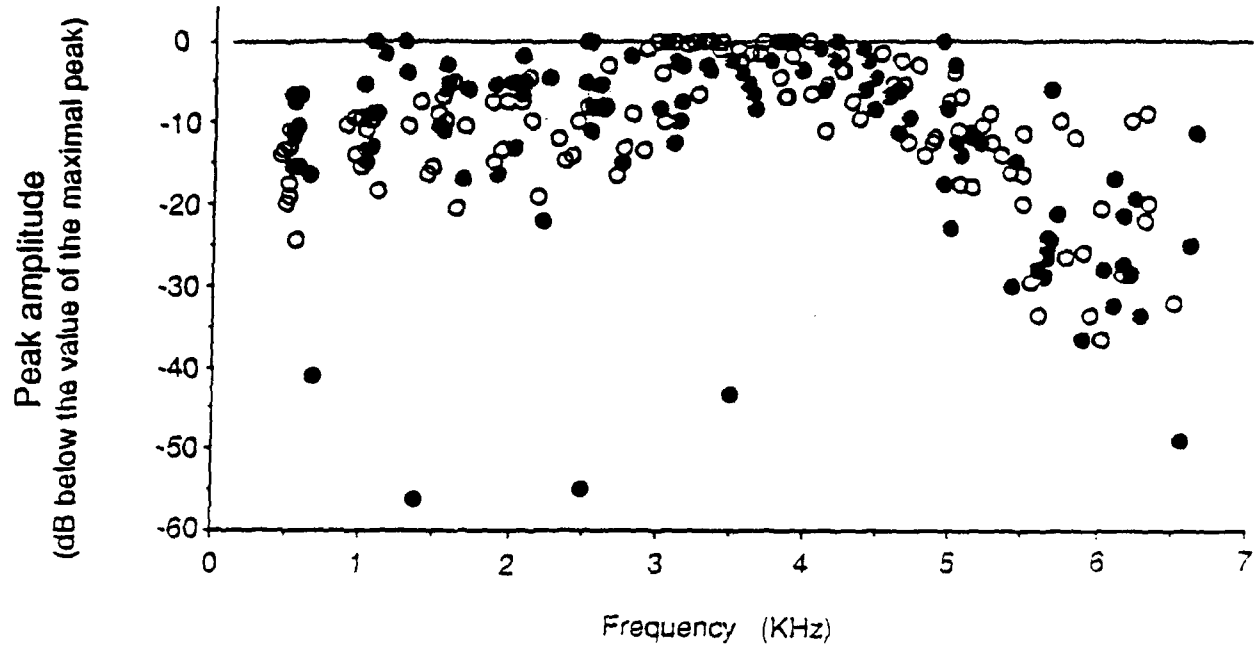
Peak amplitude
(dB below the value of the maximal peak)

Frequency   (KHz)

B

Peak amplitude
(dB below the value of the maximal peak)

Harmonic

Harmonic amplitudes in syllable 4
(dB below the value of the maximal peak)

A

r = .817   r = .681

B

100 msec

A

B

A

100 msec

B

Figure 7.

Williams, Cynx, and Nottebohm Figure 8.

100 msec

Frequency (KHz)

Frequency (KHz)
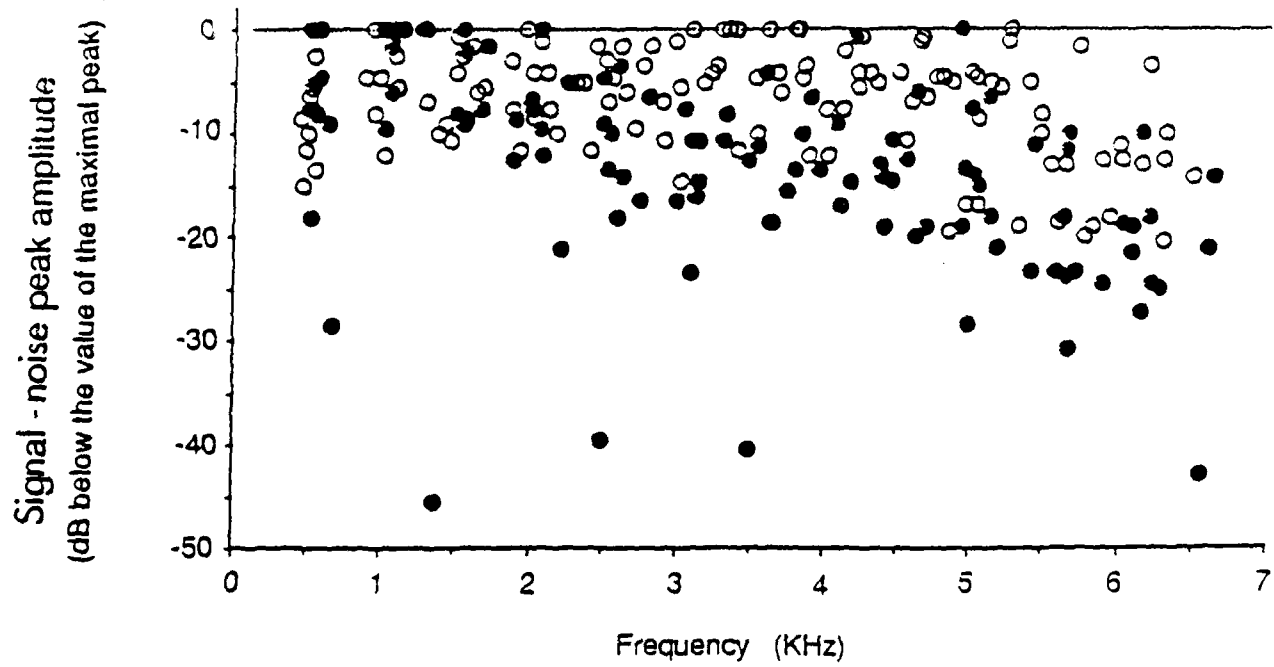
Timbre Discrimination

In Zebra Finch Song Syllables

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Field Research Center

For Ethology and Ecology

Running Head: TIMBRE DISCRIMINATION

/   Abstract

Zebra finch song syllables often vary in the relative

amplitude of individual harmonics. We determined that male

and female zebra finches could discriminate between

harmonically-structured syllables on the basis of such changes

in "timbre". Psychophysical tests showed that the

attenuation, not the increased intensity of a given harmonic

component largely controlled discrimination. The birds

memorized the frequency band important for the discrimination,

/ᵓ   and did not attend to the relative frequency characteristics

of higher or lower harmonics. Removal of amplitude or

frequency modulation in the syllable left performance above

chance, while removal of the harmonic and phase properties of

the syllable reduced performance to chance. Lesioning of

HVc, a brain nucleus essential to song production, and thought

to have a role in song perception, had no noticeable effect

on discrimination performance.

Timbre Discrimination

In Zebra Finch Song Syllables

This paper addresses the ability of zebra finches to discern subtle changes in the amplitude of harmonic components in song syllables. Zebra finch song is rich in frequency components that are harmonically related. i.e., that are integer multiples of one another. Examination of zebra finch sonograms made here and elsewhere (e.g., Immelmann, 1969; Price, 1979) showed occasional attenuated harmonics in song syllables. For example, the third harmonic in a structure of eight harmonics might be attenuated in a particular syllable. We questioned whether this fine-grained variation in song has functional significance; whether timbre may play an important role in zebra finch song, as it does in human speech.

A companion paper discusses the acoustics, learning, stability, and neural control of this naturally-occurring phenomenon (Williams, Cynx, & Nottebohm, submitted). In this paper, we show that changes in timbre of zebra finch syllables are discriminable. We then provide evidence of how such a discrimination is controlled. In Experiment 1, we trained birds to discriminate between syllables differing in which harmonic components was suppressed. In Experiments 2-5, we

established what properties of the stimuli were and were not

essential to the discrimination. In the last experiment, we

determined whether the learned discrimination was dependent'

on the hyperstriatum pars caudalis (HVc), a brain nucleus

5   essential to song production (Nottebohm, Stokes, and Leonard,

1976) and thought to play a role in song perception (Katz &

Gurney, 1979; Margoliash & Konishi, 1985; Williams &

Nottebohm, 1985).

Experiment 1: Discrimination Training

10   General Method

Subjects. Two male and two female adult zebra finches

(Poephila guttata), raised in Field Center aviaries, were

used. They were deprived of food 4-5 hours before each

session. A colony diet of mixed seed was used to reinforce

15   discrimination behavior. Water and grit were available at all

times in the home cage and test cage. When not being trained

or tested, the birds were individually caged in an aviary.

Fluorescent lighting in the aviary paralleled the natural

photoperiod.

20   Behavioral Apparatus. Four test cages were used. Each

cage was a closed cylinder of 1.8-cm wire mesh measuring 20

cm in diameter and 33 cm in length. One end of the cylinder

was mounted on a 20 x 20-cm aluminum panel that held two food

dispensers (with goal lights) mounted 10 cm apart. Only the

right food dispenser and goal light were used in the

experiments reported here. A JBL 10-cm speaker, Model 81-10H,

was centered above the food dispensers.

Perch-hopping was the operant behavior. A 22-cm aluminum

5   observation perch with a 2.5-cm infrared beam detector zone

was placed directly in front of the speaker 17 cm from the

aluminum panel. Hopping elsewhere on the aluminum perch had no

experimental consequences. Similarly equipped response perches

were placed at the entrances to the food dispensers. A wooden

10   perch placed 27 cm back from the aluminum panel allowed for

non-contingent perch-hopping.

Each test cage was placed in an IAC chamber with internal

dimensions of 36 cm high x 40 cm wide x 59 cm long. A 25-W

bulb mounted in the ceiling fixture provided illumination.

15   All inside surfaces of the chamber were lined with 1.27-cm

embossed acoustic foam. The ventilation fan was turned off

during all sessions. Behavior was observed via a one-way

glass window and a speaker monitor. Stimulus presentation,

experimental contingencies, and data collection were

20   controlled on line by an IBM AT microcomputer.

Acoustical Apparatus. A Data Translation board, Model DT-

2801 or DT-2821 digitized and produced the song syllables

(sampling rate: 20 kHz). Input was bandpass filtered (0.2 Hz,

10 kHz) before being digitized. Output went to one channel of

a BGW-85 power amplifier, a bandpass filter, then to a

speaker.

    <u>Stimuli</u>. The song syllable used in all six experiments

was edited from the song of a male zebra finch not used in

these experiments. The initial recording was made with a

Marantz cassette recorder, digitized, then edited and

resynthesized using an Fast Fourier Transform (FFT) editing

program (Zoloth, et al., 1980) implemented by Robert Dooling's

laboratory to run on a microcomputer. The second harmonic was

effectively removed from one digitized copy, the fifth

harmonic from a second copy by attenuating those portions of

the signal at least 25 dB (Figure 1). The decision to remove

these specific harmonics was based on several considerations.

First, we didn't want to remove the first harmonic, or

fundamental frequency, as that would raise questions of

periodic versus spectral pitch perception (cf., Cynx &

Shapiro, 1986). Similar questions could be raised by removal

of harmonics that are integer multiples of each other, e.g.,

the second and fourth harmonics. Second, we wanted to choose

two harmonics that were not adjacent, but were roughly equal

in amplitude. The power spectrum showed this to be so for the

second to fifth harmonics. Third, We wanted one harmonic to

be near the frequency band we had identified as containing

many of the naturally-occurring instances of attenuated

harmonics (Williams, et al., submitted). Finally, naturally-occurring instances could have been used to meet these criteria, but the artificial creation of both stimuli from the same syllable provided us with greater control over acoustic features.

Insert Figure 1 About Here

The first harmonic had a center frequency of 615 Hz, the second harmonic, 1230 Hz, the fifth harmonic, 3075 Hz, as measured on the sonogram and an FFT-based power spectrum, i.e., by these criteria, the syllable consisted of a stack of harmonics. The power spectrum showed intensity values of 52-61 dB SPL for each of the first nine harmonics. The audiogram of the zebra finch (Okanoya & Dooling, 1987) shows thresholds fairly flat (less than 5 dB variation) in the frequency range between the second and fifth harmonics and well below sound levels of individual harmonics here. We lacked a direct method of determining equal loudness, since equal contours for songbirds have not been determined. However, equal loudness contours, in humans, for example, tend to follow roughly the shape of the audiogram (Fletcher & Munson, 1933).

The difference between the two stimuli is subtle, at least to humans. We perceived a pitch at the first harmonic

for both stimuli and a slight change in timbre between
stimuli.

Procedure. The zebra finches were first trained to feed
from the food dispenser. The dispenser and its goal light were
5 automatically turned on for 20 s every 3 min. Hopping to the
center of the <u>observation</u> perch also activated the dispenser
and goal light for 20 s. All subjects initiated this operant
over 200 times within three sessions, during which the
reinforcement interval was reduced in steps to 5 s. At this
10 point, the automatic functioning of the dispenser stopped,
and a <u>go/nogo</u> procedure was instituted. Breaking the infrared
beam on the <u>observation</u> perch now produced one of the two
modified song syllables. If the syllable with the attenuated
second harmonic was played, going to the dispenser within the
15 3-s <u>go</u> period gave access to food (<u>go</u> trial). If the syllable
with the attenuated fifth harmonic was played, going to the
dispenser turned off the houselight for a 20-s timeout
interval (<u>nogo</u> trial). Failure to go during presentation of
either pattern resulted in the end of the trial after 3 s.
20 The percentage of <u>go</u> trials was reduced from 100% to a random
65%, and the reinforcement interval was reduced to 1.7 s
over the next 8-12 sessions. Each session lasted 4-6 hrs.

Treatment of Data. Acquisition and maintenance of the
discrimination were measured by recording the differences in

mean daily latencies in hops to dispenser. This measure is generally isomorphic with percent correct, but provides more information, e.g., response biases. Mean daily latencies to trials in which the fifth harmonic was attenuated (<u>nogo</u> trials) were expected to increase towards 3.0 s as the zebra finches acquired the discrimination, whereas latencies to trials in which the second harmonic was attenuated (<u>go</u> trials) were expected to remain below 1.5 s. A criterion of 1.0-s difference between daily mean latencies for three consecutive sessions was arbitrarily chosen for determining acquisition. Experimental details not described in Experiments 2-6 were identical to those listed above.

<u>Results</u>

All birds acquired the discrimination, showing similar acquisition rates. At the end of training (approximately 12 sessions or 6,000 trials), mean daily latencies on <u>nogo</u> trials were above 2.5 s. Mean daily latencies on <u>go</u> trials were at 1.5 s or below. Thus, variations in harmonic structure, similar to what appears throughout zebra finch song, and similar to those discussed in Williams, et al. (submitted), can serve as discriminative cues. We next determined the details of stimulus control.

Experiment 2: Initial Intensity Check

The discrimination could have been solved in a number of ways. The four simplest strategies might have been to listen for the presence or absence of either the second or fifth harmonic. Any one of these four strategies required attending to intensity levels of parts of the stimuli. We therefore manipulated the overall intensity levels of the stimuli to determine whether intensity did control responding.

Method

Stimuli. Go and nogo test stimuli were presented during unreinforced trials. The intensity of the test stimuli was varied from -10 dB to +10 dB with respect to the original stimulus intensity of 65 dB. This was accomplished by using the FFT editing program to alter the intensity of the entire time segment containing the syllable.

Procedure. Reinforcement remained at a random 65% of correct responses. The seven test stimuli (-10 dB, -5 dB, -2 dB, +2 db, 0 dB, +5 dB, and +10 dB with respect to the original stimulus intensity) were randomly introduced during nonreinforced trials (p=0.15). Test stimuli were collected across three test sessions. A minimum of 15 observations were collected from each subject for each data point.

Results

The birds barely responded to the nogo stimuli across the entire dB range (average latency greater than 2.98 s). They

responded to go stimuli with amplitudes below original
stimulus intensity, but stopped responding as stimulus
intensity increased from +2 dB to +10 dB. (Figure 2). There
were no significant differences between sexes, and combined
data are shown. Remember that go stimuli contained an
attenuated second harmonic. The results suggest that all four
birds were waiting for stimuli that contained little energy
at the frequency of the second harmonic, and only then
responding, and this effect was more marked the more intense
the signal. The increasing intensity of the signal added
enough energy in the formerly attenuated frequency range to
cause the birds to stop responding. This hypothesis was
directly tested in the next experiment.

Insert Figure 2 About Here

Experiment 3: Manipulation of the
Intensity of the Second Harmonic

The intensity of the second harmonic alone was
manipulated in order to determine whether it controlled
response behavior.

Method

Stimuli. We resynthesized the original song syllable so
the second harmonic varied from 15 to 2 dB below its natural

intensity level (-15 dB, -12 dB, -10 dB, -5 dB, and -2 dB).

Results and Discussion

        All four birds responded heavily to the new stimuli only
when the second harmonic approached 10 to 12 dB below its
5 natural intensity level (Figure 3). The birds could have
solved the discrimination task either by listening for the
presence or absence of either the fifth harmonic or second
harmonic. All chose to solve it by listening for the
attenuated second harmonic.

                ----

10   Insert Figure 3 About Here

        The intensity of the fifth harmonic appeared to have no
effect on performance. For example, test stimuli in Experiment
3 possessed an non-attenuated fifth harmonic. However, they
were treated as similar to the baseline stimuli with an
15 attenuated fifth harmonic when the second harmonic intensities
were similar.

        The next two experiments addressed the contextual
properties of the acoustic stimulus required to maintain the
discrimination. We question_ whether the stimulus control
20 exerted by the presence or absence of the second harmonic was
itself dependent on other properties of the stimulus. For
example, was it necessary to recognize the frequency

information as the second harmonic per se, or simply as a relatively low frequency band (Experiment 4)? And was there other contextual information in the stimulus, possibly affected by our editing procedures, that controlled behavior (Experiment 5)?

Experiment 4: Manipulation of Individual Harmonics

The birds may have acquired a high/low relative pitch discrimination in which the removal of any frequency information below a certain frequency was the discriminative cue. If so, the response given to the attenuated second harmonic should generalize, e.g., to attenuated first or third harmonics. Alternatively, they may have acquired an absolute pitch discrimination, memorizing the frequency location of the second harmonic. Removal of other individual harmonics then would have little or no effect on the discrimination performance.

The question of relative versus absolute pitch perception in songbirds has been addressed elsewhere (Cynx, Hulse, & Polyzois, 1987). Absolute pitch perception appears ubiquitous in songbirds, but no clear evidence exists for relative pitch perception. This experiment raised the same issues by determining whether the second harmonic alone controlled discrimination performance.

Method

Stimuli. Nine test stimuli were synthesized with
harmonics 1-9 attenuated 25-30 db respectively. A tenth test
stimulus had no harmonics attenuated (the prototypical
syllable).

Results

The birds responded only when the second harmonic was
attenuated. No apparent generalization gradient across
frequencies was noted. They also failed to respond to
presentations of the full harmonic structure (Figure 4). The
birds had memorized the attenuated second harmonic, at least
to the precision of the 615 Hz distance between each harmonic.
They did not employ a relational high-low strategy on which
any attenuated harmonic below, e.g., the fifth harmonic was a
cue to respond.

Insert Figure 4 About Here

It is possible the birds were attending to the second
harmonic as a harmonic, and that correct responses to stimuli
could be maintained if the entire stack of harmonics shifted
along a log 2 scale, thereby preserving the harmonic ratios
between frequency components. Relative pitch perception may
be dependent on frequencies being embedded in a harmonic
structure (Cynx & Shapiro, 1985). However, to a first

approximation, the results here provide another instance in which songbirds choose to employ absolute rather than relative pitch information.

Experiment 5: Removal of Frequency Modulation,

Introduction of Sinewave Stimuli,

and Removal of Harmonic Information

This experiment was undertaken to ensure that stimulus control was tied to the frequency components of each harmonic alone. Both the beginning and end of the syllable showed some frequency modulation (Figure 1). Perhaps the birds were attending to a non-frequency property of the signal that inadvertently occurred in the region of the second harmonic. We therefore removed time segments that contained the frequency modulation. Second, we replaced the entire stimulus with a set of sine waves that matched the harmonic structure of the syllable. This was a more drastic test to see if discrimination was controlled by non-harmonic elements in the signal. Finally, we removed all harmonic information, but left the frequency gap around 1230 Hz. This was the strongest test to see if the lack of energy alone was the discriminable cue.

Method

Stimuli. First, we removed the frequency modulated time segments of both training stimuli. The front and back of the training stimuli (100 ms) each were clipped by 10 ms. A 5-ms

cosine function was then placed on the front and back of the
remaining signal to prevent onset and offset clicks. Second,
we created go and nogo test stimuli with sine waves at the
center frequencies of the necessary first eight harmonics.
_5_   These stimuli were digitally synthesized so that they were
the same length as the training stimulus and had 5-ms
rise/fall functions. All harmonics were equal in intensity
and started in phase with each other. Overall intensity was
65 dB(A). Finally, we edited a 100-ms band of white noise
_10_   (range of 0.2 kHz to 8 kHz) to create a pair of stimuli with
missing frequency ranges matching the training stimuli (a
frequency gap of approximately 800 Hz centered around 615 Hz
or 3075 Hz). Again, the test stimuli had 5-ms rise/fall
ramps, and intensity was set at 65 dB(A).

_,5_   Procedure. Each of the three test conditions was run for
one session, with the two given test stimuli randomly
introduced during nonreinforced trials (p=0.15).

Results

The results show the necessary contexts in which the
_20_   absence or presence of the second harmonic controlled
behavior. Removal of frequency modulation resulted in no
noticeable differences between test and training stimuli
(Figure 5, top). The additional removal of amplitude
modulation also failed to destroy discrimination completely

(Figure 5, middle); the increase in mean go latencies between

the training and synthetic sine wave stimuli was due entirely

to one of the male birds failing to maintain the

discrimination (Figure 5, middle). However, all four birds

5   failed to maintain the discrimination between the white noise

stimuli (Figure 5, bottom).

Insert Figure 5 About Here

Experiment 6: Lesioning of Brain Nucleus HVc

Hyperstriatum pars caudalis (HVc), a brain nucleus

10 essential for the production of learned song (Nottebohm, et

al., 1976), also responds to acoustic stimulation (Katz &

Gurney, 1981; Margoliash, 1983; Margoliash & Konishi, 1985).

Sounds played to male zebra finches induce activity in HVc,

that then drive neurons down to the level of the motor nucleus

15 controlling the syrinx. It has been hypothesized that this

activity then finds its way back to the forebrain (Williams

& Nottebohm, 1985). Why is acoustic information being

monitored and processed by the production nuclei in adult

songbirds? Perception and production processes in songbirds

20 may encompass highly integrated neural circuitry, such that

perception of song elements is dependent on this circuitry.

We tested this hypothesis by measuring discrimination

performance to our stimuli before and after HVc lesions.

Method

        Procedure. Baseline performance was collected for three

5   days before the operation for all four birds. Reinforcement

was held at a random 65% of correct responses. Bilateral

electrolytic lesions then were performed on a male and female

on day 4. No birds were run till day 7 to allow for recovery.

Subjects then were tested on baseline performance for the

10 next 3 days.

        Histology. After the completion of testing, the lesioned

birds were perfused with formol saline. The brains were

removed and section on a vibratome (50 μm), mounted, and

stained with cresyl violet. The extent of the lesions was

15 then ascertained. Both HVc of both birds had been severely

damaged (95-100% of volume) or removed. Some disruption of

underlying areas, including the shelf region (see Kelley &

Nottebohm, 1979) and of the dorsal portions of Field L, the

avian primary auditory projection to the forebrain, may have

20 occurred.

Results

        Bilateral lesioning of HVc had no discernible effects

on the discrimination (Figure 6). The mean nogo and go

latencies for the lesion pair were 2.97 and 1.68 for the

three sessions before lesioning and 3.00 and 1.61 for the
three sessions after lesioning. Means for the control pair
were 2.97 and 1.77 before and 2.95 and 1.85 after. A more
detailed examination of performance within sessions also
failed to uncover differences. There were no differences
between groups when comparing mean latencies of the first 20,
50, or 100 trials in sessions.

Insert Figure 6 About Here

General Discussion

Harmonic and Coherent Structures

We have shown that zebra finches can use changes in
timbre to discriminate between song syllables. In
discriminating between the stimuli used in this research, the
zebra finches consistently used the lack of energy in a
particular frequency band as the discriminative cue. And they
appeared to have memorized this frequency location. However,
a band of white noise with roughly the appropriate gap in
sound around the second harmonic failed to maintain the
discrimination. Frequency information appeared to control
performance only when it was embedded within the harmonic
structure. The results suggest that the zebra finches may
have been attending to energy levels at a given harmonic

rather than a given frequency. However, we have no direct
evidence to support the inference that zebra finches heard
the harmonics of the stimuli as harmonics. The white noise
stimulus (Experiment 5) altered other information besides
5   harmonic information. For example, phase relations between
frequency components were randomized by white noise. We do
not know if such phase information is perceptibly salient to
birds. Traditionally, humans have been thought to be phase
deaf (Helmholtz, 1885/1954). However, recent evidence suggests
10   otherwise. When parts of a signal have similar time-amplitude
envelopes, or show coherence, phase can affect the perceptual
salience of components within the stimulus (Hall, 1984). A
further analysis involving phase information would not only
raise specific hypotheses as regards perception, but may also
15   place constraints on the sorts of mechanisms producing the
song.

Periodicity Pitch Perception in Songbirds and Humans

Humans have not yet been carefully tested to determine
how they would perceive the stimuli used in these experiments.
20   However, informal testing suggested the 615 Hz periodic
property of the stimuli produced a 615 Hz pitch. One then had
to concentrate on the timbre to hear the differences between
the stimuli with the attenuated second or fifth harmonic. It
is unclear whether the birds also heard the pitch of the

first harmonic. Starlings, a species of songbird, possess

periodicity pitch perception (Cynx & Shapiro, 1986). However,

this perception was revealed only when the birds were placed

on a forced-choice task. Otherwise, the birds showed a

5 preference for using absolute frequency information (Cynx,

unpublished data), much as was seen in these experiments.

Humans and songbirds may be biased differently in what

acoustic cues to employ in discriminating between such complex

frequency structures. We are currently engaged in testing

10 this comparative question.

Attenuated Harmonics and Formants

Why the birds chose to employ the second rather than the

fifth harmonic as the discriminative cue remains an open

question. The necessary tests for directly determining whether

15 the attenuated second harmonic controlled discrimination

strictly because of its perceptual or behavioral properties,

such as reversing the go and no/go contingencies for the

stimuli, have not been done. However, there are reasons to

suspect that perceptual factors played an important role.

20 The attenuated second harmonic that controlled performance

was closer to the frequency band that contains a

disproportionate number of naturally-occurring attenuated

harmonics (Figure 9a, Williams, et al., submitted). The first

and third harmonics were the mostly frequently suppressed,

while the range of suppressed individual harmonics and
patterns of harmonics varied from the first to the tenth
(Table 2, Williams, et al., submitted). Thus, the most obvious
production constraint appears to be that lower order harmonics
are suppressed with a markedly higher frequency in the
population of birds examined. We suggest two perceptual
reasons for this constraint. First, certain frequency regions
may have special salience to the zebra finches, analogous to
the special salience of formants and loci in human speech
(Liberman, et al., 1967). The fact that the salient
information is silence rather than sound here could simply
mean that the zebra finch vocal tract functions inversely by
producing perceptible 'anti-formants' rather than perceptible
formants. A second suggestion, based on what is known about
peripheral auditory processing, is that lower-order harmonics
may be more perceptible (especially given the AM present in
the signal) because of the change in critical ratios across
the frequency ranges for the harmonics (Okanoya & Dooling,
1987). The seeming perceptual equality of the harmonics
typically displayed on a sonogram (e.g., Figure 1) fails to
account for known psychoacoustic properties of the peripheral
auditory system.

<u>Song Perception and Brain Pathways for Song Production</u>

Perceptual processing of song could be subsumed under general acoustic processing. Alternatively, it could require special neural integration between mechanisms used in hearing and producing song. The question is whether we can provide essential behavioral tests for such special processing. The neurophysiological findings, as suggestive as they may be (Katz & Gurney, 1979; Margoliash, 1985; Williams & Nottebohm, 1985), cannot provide evidence of a strictly perceptual nature.

Experiment 6 directly tested one of the most general hypotheses: Does the accurate discrimination of song syllables by adult zebra finches universally require processing by the song production pathway? Apparently not. Discrimination of any given song syllable, based on a naturally varying feature, may not require song production circuitry. The results were foreshadowed to some degree by results of the first five experiments. Female zebra finches have a much smaller HVc (Nottebohm & Arnold, 1976), and lack some of the circuitry essential to song production (Konishi & Akutagawa, 1985, Williams, 1985). Yet the females showed no performance differences in acquiring or maintaining the discrimination.

The results suggest a number of more constrained hypotheses. Invocation of special processing may require the embedding of a syllable in full song. Or it may require

discrimination of some unknown categorical boundary between

syllable variants. It may be that the task can be solved by

either general auditory processing or special processing, and

that our measure of discrimination performance was not fine-

5 grained enough to detect differences in processing (cf.

Whalen & Liberman, 1987). For example, songbirds might be

expected to notice numerous differences between song syllables

they hear well before the exemplars are repeated thousands of

times. And we note that lesions here were performed _after_

10 acquisition of the discrimination task. Perhaps the role of

HVc is critical in acquisition of such a discrimination

rather than in its maintenance.

Experiment 6 in particular was the first step in

attempting to provide a behavioral measure of birdsong

15 perception that will correlate with a number of

neuroanatomical and neurophysiological findings. We have yet

to find evidence that the perceptual processing of birdsong

differs from general acoustic processing. However, a

satisfactory explanation of how birds process conspecific

20 vocalizations will not be possible until we have specified

the levels at which patterns of production and perception

do correlate with each other. As this paper and the companion

study (Williams, et. al, submitted) show, zebra finch song

contains features both prevalent and discriminable at the

level of fine-grained acoustic analysis. We presume the same
holds true for many other songbirds.

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Author Notes

Figure Captions

Figure 1. The two training stimuli, built by attenuating either
the second and fifth harmonics at least 25 dB to produce the go
(second harmonic attenuated) and no/go (fifth harmonic
attenuated) stimuli respectively. The stimuli were 100 ms in
duration each. Horizontal lines mark off 1 kHz frequency bands
(Kay sonograph, wide-band filter (300 Hz), without use of Hi-
shape filter).

Figure 2. Results as the intensity of both go and nogo stimuli
were varied in intensity. Open circles show go stimuli
(attenuated second harmonic). Solid circles show nogo stimuli
(attenuated fifth harmonic). The dependent variable is latency
to respond within the 3-s go period. Each data point is the
mean of the daily means for four birds, with at least 15 data
points for each bird. Error bars show standard errors of mean.

Figure 3. Results as intensity of second harmonic varied from -
-15 to -2 dB below its natural level. Baseline performance for
go stimuli (attenuated second harmonic) is shown at far left.
Baseline performance for nogo stimuli (attenuated fifth
harmonic) is shown at far right.

Figure 4. Results as harmonics 1-9 were removed respectively.
The circle on the far left shows responses to the prototypical
stimulus that included all nine harmonics.

Figure 5. Results as frequency modulation was removed from the
stimuli (top), amplitude modulation was removed from the signal
(middle), and all information except the attenuated energy in
the region of the second   fifth harmonic was removed from the
signal (bottom). Open ci   show go stimuli (attenuated
second harmonic). Solid circles show nogo stimuli (attenuated
fifth harmonic).

Figure 6. Pre- and post-experimental results (Days 1-3 and 7-9
respectively) for birds with lesions (top) or no lesions
(bottom) to HVc. Circles identify females, squares identify
males. Open shapes show go stimuli (attenuated second harmonic).
Solid shapes show nogo stimuli (attenuated fifth harmonic).

Lesion

Control