

# Conspecific and Heterospecific Song Discrimination in Male Zebra Finches with Lesions in the Anterior Forebrain Pathway

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**ABSTRACT:** Adult zebra finches can produce normal song in the absence of Area X, IMAN, or DLM, nuclei that constitute the anterior forebrain pathway of songbirds. Here, we address whether lesions involving Area X and IMAN affect adult male zebra finches' ability to discriminate between conspecific or heterospecific songs. Intact birds and lesioned birds were trained on an operant GO/NOGO conditioning paradigm to discriminate between hetero- or conspecific songs. Both lesioned and intact birds were able to learn all discriminations. Lesioned and intact birds performed equivalently on canary song discriminations. In contrast, discriminations involving bird's

own song took significantly more trials to learn for lesioned birds than for intact birds. Discrimination between conspecific songs in general also took longer in the lesioned birds, but missed significance level. Birds with control lesions medial to Area X did not show any differences from intact animals. Our results suggest that an intact anterior forebrain pathway is not required to discriminate between heterospecific songs. In contrast, Area X and IMAN contribute to a male zebra finch's ability to discriminate between its own song and that of other zebra finches. © 1998 John Wiley & Sons, Inc. *J Neurobiol* 36: 81–90, 1998

## INTRODUCTION

The song system of adult male zebra finches and other song birds consists of a series of anatomically discrete nuclei (Nottebohm et al., 1976, 1982; Okuhata and Saito, 1987; Bottjer et al., 1989; Vates et al., 1997) arranged into two pathways: an efferent pathway necessary for the production of learned song, and an anterior forebrain (AF) pathway necessary for song learning (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991). Both pathways originate at the High Vocal Center (HVC) (Fig. 1). HVC projects to the robust nucleus

of the archistriatum (RA). RA in turn projects to the tracheosyringeal portion of the hypoglossal nucleus (nXIIIts), which innervates the muscles of the vocal organ, the syrinx (Nottebohm et al., 1976). The AF pathway provides an alternate, indirect route from HVC to RA. In this circuit, HVC projects to Area X of lobus parolfactorius, which projects to the medial nucleus of the dorsolateral thalamus (DLM). DLM projects to the lateral magnocellular nucleus of the anterior neostriatum (IMAN), which in turn projects to RA and also back onto Area X (Nixdorf-Bergweiler et al., 1995; Vates and Nottebohm, 1995).

The function of Area X, IMAN, and DLM in adult zebra finches is enigmatic: In adulthood, normal song production is apparently not affected by lesions in this pathway (Bottjer et al., 1984; Halsema and Bottjer, 1992; Sohrabji et al., 1990; Scharff and Nottebohm, 1991; Nordeen and Nordeen, 1993); yet, singing can induce electrophysiological activity (Hessler and Doupe, 1997) and im-

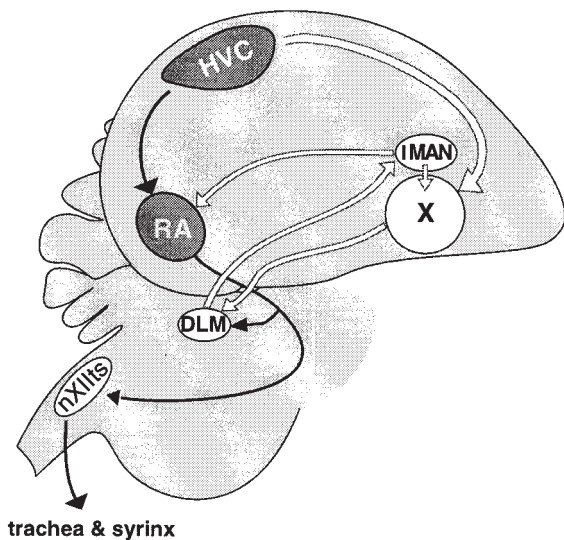
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**Figure 1** Saggital section through adult song bird brain showing the relation of Area X and IMAN to other song system nuclei and the layout of the afferent (black arrows) and anterior forebrain pathways (white arrows).

mediate-early gene expression in those nuclei (Jarvis and Nottebohm, 1997).

What, then, is the role of these nuclei in adult male zebra finches? Here, we consider the possibility that the AF pathway plays a role in song perception. Neuronal units that respond selectively to playbacks of conspecific song in general and particularly to playbacks of the bird's own song (BOS) are well represented in the nuclei of the AF pathway (Doupe and Konishi, 1991; Doupe, 1997) as well as in the motor pathway (Margoliash, 1983, 1986; Williams and Nottebohm, 1985; Doupe and Konishi, 1991; Margoliash and Fortune, 1992; Vicario and Yohay, 1992). The selectivity for BOS is acquired as a bird learns its own song (Volman, 1993; Doupe, 1997). There are other lines of evidence suggesting that BOS has special status in a bird's perceptual world: In intact male zebra finches, discriminations involving BOS are by far the easiest to master (Cynx and Nottebohm, 1992a; Uno and Maekawa, 1997). Field playback experiments suggest that birds perceive their own song as different from other stimuli (Falls et al., 1988; MacArthur, 1986). Moreover, analysis of song matching in great tits, song sparrows, and western meadowlarks suggests that birds use their own song as a standard when classifying the songs they hear (Horn and Falls, 1996).

We tested whether the AF pathway needed to be intact for zebra finches to successfully discriminate between heterospecific songs, between conspecific songs, and between the BOS and another conspe-

cific song. The results we report clearly show that discriminations involving the BOS were the hardest to learn for birds with AF lesions. Other conspecific song discriminations were slightly less difficult, while discriminations among canary songs and a stimulus-reversal task were learned equally well by intact and lesioned animals.

## MATERIALS AND METHODS

### Subjects

Sixteen adult male zebra finches (*Taeniopygia guttata*; older than 120 days), bred and raised at the Rockefeller University Field Research Center, were arbitrarily divided into eight pairs. One bird in each of six pairs received lesions targeted at Area X, and one bird in each of the two remaining pairs received control lesions. Members of a pair were housed in a cage divided by a wire screen, when not being trained or tested. Water and grit were available at all times. Seed was withheld 7–9 h before testing began. All birds were on a 12:12 h light/dark photoperiod with full-spectrum lighting.

### Surgery and Histology

One bird in each pair was selected arbitrarily and given a bilateral electrolytic lesion stereotaxically targeted at Area X, following previously reported procedures (Scharff and Nottebohm, 1991).

Birds were perfused with 60 cc each of phosphate-buffered saline and 4% paraformaldehyde in 0.1 M phosphate buffer under deep anesthesia induced by methoxyflurane (Metofane; Pitman-Moore), followed by injection of 0.03 mL each of ketamine (Ketalar; Parke-Davis) and xylazine (Rompun; Haver). Brains were removed and stored in paraformaldehyde solution. Next, 50- $\mu$ m vibratome sections were cut in the frontal plane, mounted, and stained with 0.1% solution of Cresyl violet acetate (Sigma). Volumes of Area X in both intact and lesioned animals were calculated by measuring the areas of the nucleus on a computer-interfaced microscope (Alvarez-Buylla and Vicario, 1988) in all sections that contained the nucleus and multiplying the sum of areas by the thickness of the sections. Area X was measured in both hemispheres, then averaged. The effectiveness of lesions targeted at Area X was expressed in each bird as a percentage of the mean volume (1.663 mm<sup>3</sup>) of Area X in eight intact adult animals.

To check whether lesions targeted at Area X had affected the integrity of IMAN, three experimental birds also received bilateral injections of the retrograde tracer Fluorogold (Fluorochrome) into RA 5 days prior to sacrifice. Sections were then examined under ultraviolet (UV) illumination for the presence of retrogradely labeled neurons in IMAN, and volumes were reconstructed from measurements (as de-

scribed above for Area X) around the retrogradely labeled cells. In the three other Area X-lesioned birds, damage to IMAN was assessed in Cresyl violet-stained material as described above for Area X, i.e., by comparing IMAN volume in lesioned animals to the mean value of IMAN measured in eight intact birds (0.0929 mm<sup>3</sup>).

Song directed at a female was recorded before surgery as well as 2 and 56 days after surgery. As in an earlier study (Scharff and Nottebohm, 1991), no obvious changes in song production were observed.

## Operant Setup

Operant techniques have been previously used to search for the cognitive properties underlying sound perception in birds and how birds process conspecific and hetero-specific vocalizations (e.g., Dooling, 1992; Hulse et al., 1984; Cynx et al., 1990, 1992; Cynx and Nottebohm, 1992a,b). Six identical operant stations (described in Cynx et al., 1990) were used [Fig. 2(A)]. One wall of the test cage contained a food dispenser with a goal light and speaker. An observation perch was placed in the middle of the cage and a second (response) perch was placed in front of the food dispenser. The operant behavior was hopping from the observation perch to the response perch. Both perches were equipped with infrared detectors to monitor perch occupancy. Because zebra finches are generally very active, a third perch was placed at the end of the cage farthest from the feeder, so that the bird could hop between some perches without this leading to experimental consequences (Stevenson, 1967; Cynx et al., 1990). The test cage was placed inside a sound attenuation chamber lined with acoustic foam. A 25-W light provided illumination, and behavior could be observed via a one-way glass window and speaker monitor. Stimulus presentation, experimental contingencies, and data collection were controlled by a microcomputer running custom-made software. Song segments used as stimuli lasted approximately 800 ms. Each zebra finch song segment consisted of a song motif (Sossinka and Böhner, 1980). Song stimuli were recorded on a cassette tape, then digitized using a 12-bit digital/analog board at a sampling rate of 20 KHz. The overall loudness of each stimulus was set to 70 dB (A) SPL as measured at the observation perch. The stimuli were played through the digital/analog board. Output went to the speaker in the test cage via a power amplifier and a bandpass filter (0.2–10 kHz).

## Test Stimuli and Behavioral Procedures

To determine the effect of lesions on auditory discriminations, each pair of birds was tested on five consecutive discrimination tasks after initial training to master the operant procedure (see Cynx and Nottebohm, 1992a, for detailed experimental design). In the initial training phase (2 weeks or more postsurgery), birds were trained to work for a seed reward. A bird that went to the observation perch of the test chamber and then moved within the next 3 s to the response perch was rewarded with access

to seed. Intact and lesioned birds learned this procedure equally well. The paradigm is summarized in Figure 2.

The first discrimination task was between two Waterschlager canary (*Serinus canaria*) song segments. Four different pairs of stimuli were used for the six pairs of birds. This task taught the birds to use sound stimuli as indicators of access to food. It also determined whether the lesions interfered with a bird's ability to learn to "play the game," i.e., to discriminate between two segments of heterospecific bird song through the following routine: A bird initiated a trial by moving to the observation perch. The computer randomly presented a stimulus that required a GO or NOGO response. A GO response consisted of moving to the response perch within a 3-s GO period. A GO response to the GO stimulus lit the goal light and produced access to food [Fig. 2(B)]. A GO response to a NOGO stimulus turned off the cage light for 15 s, signaling to the bird that it had made an error [Fig. 2(C)]. A NOGO response, i.e., the bird not going to the response perch, always resulted in the end of the trial after 3 s. Sessions lasted 3–4 h. The number of trials required to acquire the discrimination was used to judge the difficulty of the learning task. Analysis of the acquisition was performed by grouping data in blocks. A score of 75% correct responses across a block of 100 trials was used as the learning criterion (Cynx and Nottebohm, 1992a; Cynx et al., 1992).

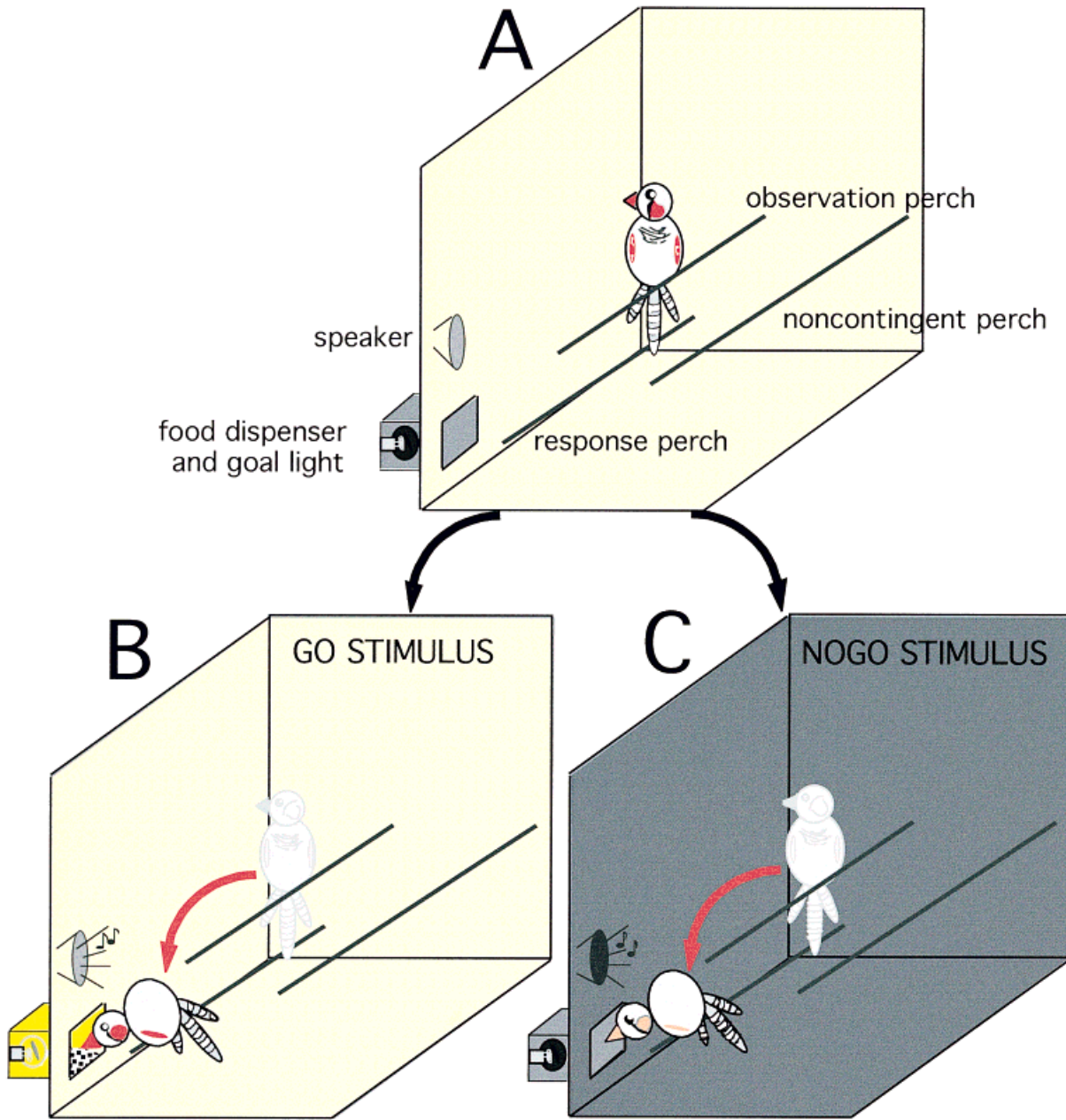
The session after a bird had reached the learning criterion, it was presented with the second discrimination task in which the canary song segments were replaced with zebra finch song stimuli. For each pair of stimuli, the GO stimulus was the BOS; the NOGO stimulus was the song of his cagemate. The third discrimination task reversed the stimulus–response contingencies so that the GO stimulus of the previous task became the NOGO stimulus, and vice versa. The fourth task required that the bird discriminate between two unfamiliar zebra finch songs, and the fifth and last task presented the birds with two novel canary song segments. All zebra finch songs recorded for stimulus tapes were directed toward females.

## RESULTS

### Histology

Table 1 summarizes the sites and relative sizes of the lesion: The lesions targeted at Area X [Fig. 3(B)] ablated 45–100% of intact Area X [Fig. 3(A)] and were of comparable size in both hemispheres. Juvenile male zebra finches that receive lesions of this magnitude produce grossly aberrant song as adults (Scharff and Nottebohm, 1991). Lesions targeted at a region medial to Area X [Fig. 3(C)] were of equivalent size, but barely touched Area X itself, and thus left 80% or more of Area X intact. LMAN was not affected in the control birds.





**Figure 2** (A) Operant conditioning test cage. (B) For a GO response to a GO stimulus, the bird needed to move to the response perch within 3 s. This activated the goal light and produced access to food. (C) A GO response to a NOGO stimulus turned off the cage light for 15 s, signaling to the bird that he made an error. No food reward was produced. A NOGO response (not shown) always resulted in the end of the trial after 3 s.

In the experimental group, IMAN's integrity was also compromised, but to a lesser degree than Area X. The sizes of Area X lesions covaried strongly with the sizes of IMAN lesions ( $n = 8$ ;  $R = 0.922$ ;  $R^2 = 0.851$ ;  $p = 0.0011$ ). Therefore, we cannot separate the respective contributions of the Area X versus the IMAN lesions, even though the lesions

compromised Area X's integrity considerably more than they did IMAN. We use the term "AF-lesions" to reflect this circumstance.

### Behavior

The five consecutive discrimination tasks that birds were exposed to apparently differed in difficulty

**Table 1** Location and Sizes of Lesions in Area X and IMAN

Bird	Area X			IMAN			Mean Area X/IMAN		
	Right	Left	Mean	Right	Left	Mean	Right	Left	Mean
blk27	100	100	100	66	83	75	83	91	87
y25	100	100	100	61	61	61	80	80	80
mag2	100	100	100	10	100	55	55	100	78
red64	100	100	100	34	44	39	67	72	70
dg62	46	80	63	3	53	28	24	67	45
dg1	64	26	45	0	11	6	32	19	25
Group means	<b>85</b>	<b>84</b>	<b>85</b>	<b>29</b>	<b>59</b>	<b>44</b>	<b>57</b>	<b>71</b>	<b>64</b>
dg86	49	7	28	0	0	0	24	3	14
mag1	7	21	14	0	0	0	3	10	7
Group means	<b>28</b>	<b>14</b>	<b>21</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>14</b>	<b>7</b>	<b>10</b>

Data are expressed as average volume of six intact animals, in experimental (top) and control (bottom) lesioned birds. For each bird, left hemisphere, right hemisphere, and their average are listed. The last category, “mean Area X/IMAN,” lists the average of percent Area X and percent IMAN lesioned.

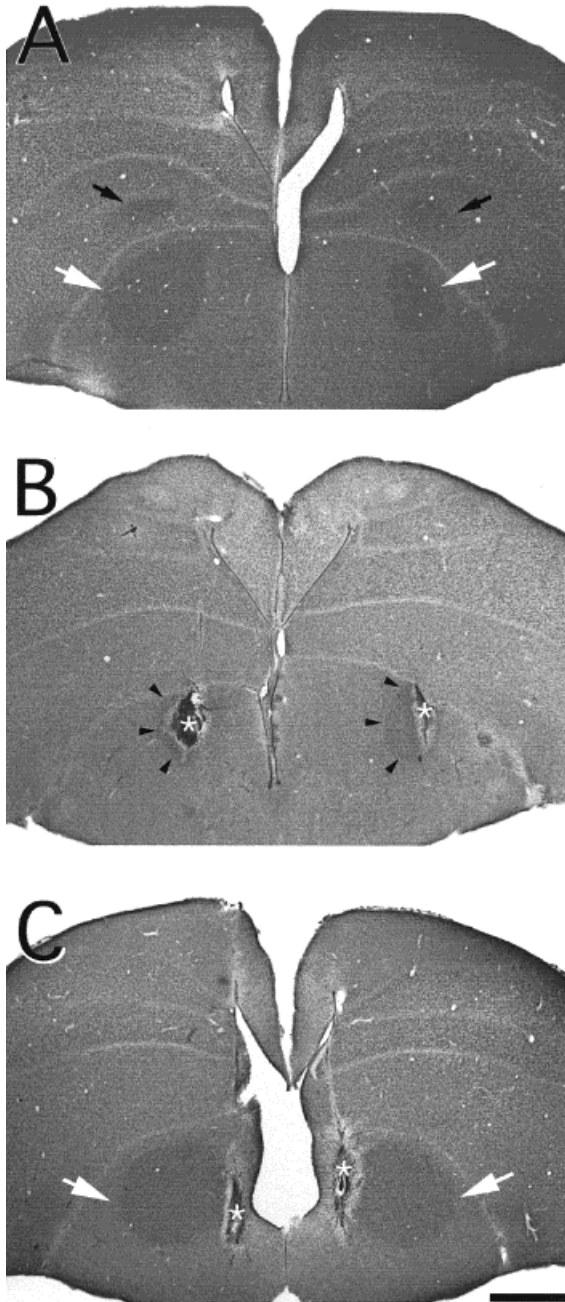
(Fig. 4). All birds needed almost 2000 trials before they were able to reliably differentiate between the canary songs in task 1. However, once birds had learned the procedure, the new canary songs presented in the last tasks were as easy to discriminate as conspecific songs in tasks 2 and 4, all of which were mastered in a few hundred trials.

The only task in which AF lesioned birds differed by more than twofold was task 2 [Fig. 4(A)]; in fact, inspection of the performances of individual pairs across tasks revealed that this discrimination was the only task in which every one of the six experimental AF lesioned birds needed more trials than their intact partners. Statistical analysis of the performance on task 2 (described below) indicated that discrimination between BOS and that of a cagemate was significantly influenced by the presence of a lesion in the AF pathway ( $p < 0.01$ ). In contrast, there was no difference between control lesioned and intact birds on this task ( $p > 0.10$ ).

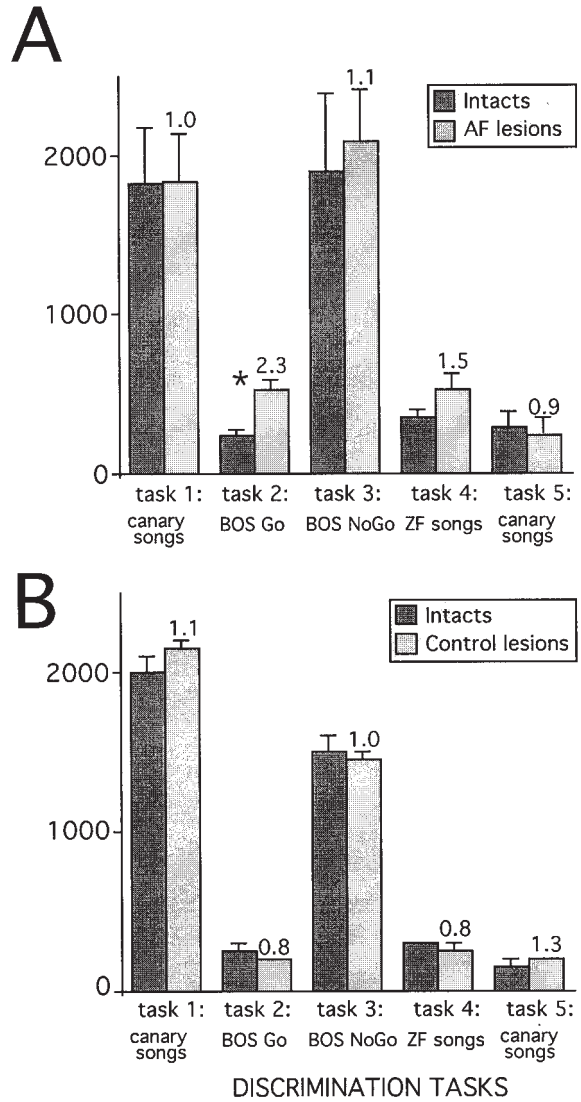
In addition to the marked variability across tasks in number of trials needed to reach criterion, there also was variability between individuals performing the same task [Fig. 4(A)]. This variability might have resulted from differences in the difficulty of discriminating different pairs of sounds (see Methods). An  $F$ -maximum test for homogeneity of variance (Bruning, 1977) showed that the variances were in fact not homogeneous. To run an analysis of variance (ANOVA) that focused on the effects of the lesions on discriminations on any one task and across tasks without the confounding factor of variability due to other factors, we decided to express the data of each lesioned bird in reference to

its intact partner. This normalization was achieved by dividing the number of trials to criterion for both birds in a pair by the number of trials for the intact bird. A repeated-measures ANOVA using the presence or absence of lesion as an independent measure and the normalized trial data as a dependent measure showed that there was a significant group difference between the AF-lesioned ( $n = 6$ ) and intact animals ( $n = 6$ ) [ $F(1, 10) = 7.909$ ;  $p = 0.0184$ ]; to find out which tasks contributed to this group difference, we used a Tukey honest significant difference test for post-hoc comparison of means. This showed that the group difference was significant only in task 2, which required discrimination between BOS and that of a cagemate ( $p < 0.01$ ). The repeated-measures ANOVA also showed a significant effect across tasks [ $F(4, 40) = 4.282$ ;  $p < 0.0056$ ]. In this case, the Tukey test showed that the results of the lesioned group in task 2 were significantly different from results in all other tasks ( $p < 0.01$ ). The ANOVA for control lesioned animals ( $n = 2$ ) and their intact partners ( $n = 2$ ) did not show any significant differences either between groups [ $F(1, 2) = 0.096$ ;  $p = 0.786$ ] or across tasks [ $F(4, 8) = 1.398$ ;  $p = 0.3176$ ].

Analyzing the performances of individual birds across the different tasks revealed that there were no consistently “slow” or “fast” learners, so that performance on one task did not generally predict the performance on another. There was, however, one interesting exception: In the lesioned group, the performance on task 2 (BOS vs. song of cagemate) strongly correlated with the performance on task 3, in which the stimulus–response contingencies were reversed ( $n = 6$ ;  $R = 0.931$ ;  $R^2 = 0.868$ ;  $p = 0.007$ ;



**Figure 3** Photomicrographs of frontal, Cresyl violet-stained, 50-µm tissue sections. (A) An intact bird, showing the pear-shaped Area X (white arrow) and IMAN (black arrow) (B) Bilateral lesion (white asterix) targeted at Area X. Some unlesioned Area X tissue is visible medial to the lesion in the right hemisphere and lateral to the lesion in the left hemisphere. The total lesion of this particular bird was 63% of the average Area X in intact animals. (C) Bilateral lesions (white asterix) medial to Area X (white arrows). The lesion encroaches slightly on the medial border of Area X.

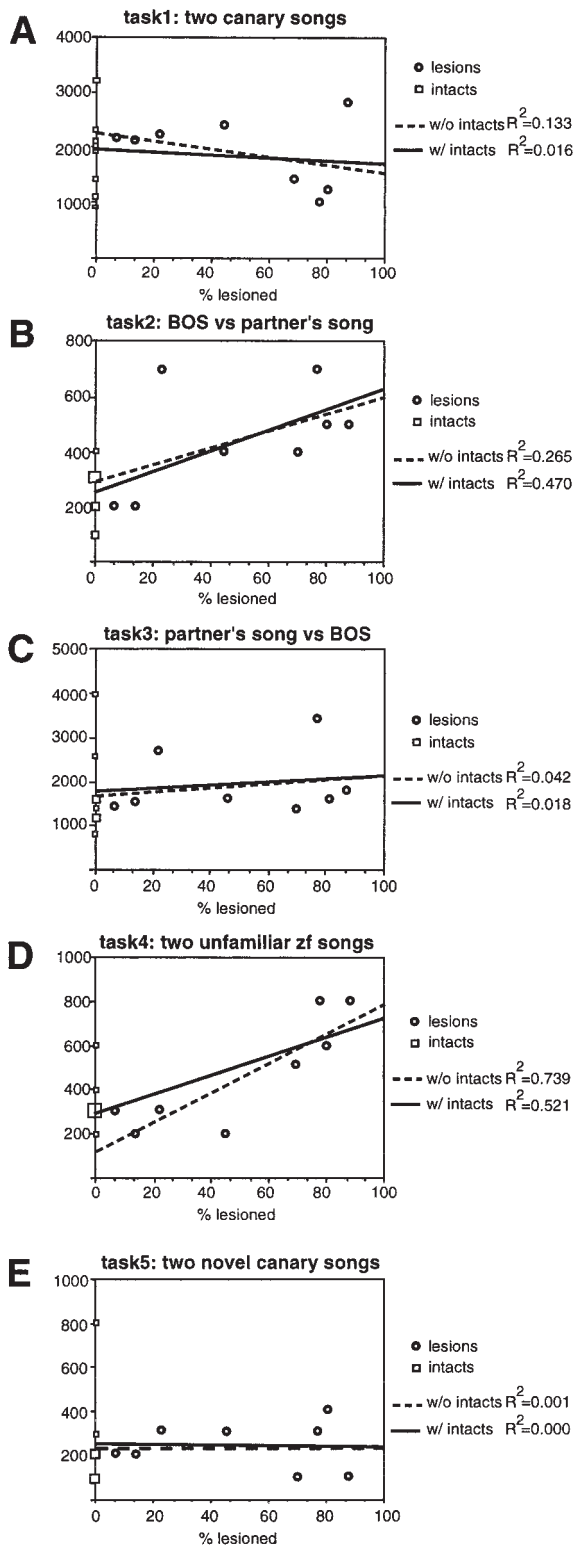


**Figure 4** Learning performance by the two experimental groups of zebra finches on the five discrimination tasks (*x* axis). The *y* axis shows trials to criterion. Dark gray bars and S.E.M.s represent intact birds; light gray bars and S.E.M.s represent birds with lesions to Area X/IMAN (A) or birds with control lesions medial to Area X (B). The number over each lesioned bar indicated the fold increase of lesioned over intact birds.

if control lesions included  $n = 8$ :  $R = 0.832$ ;  $R^2 = 0.692$ ;  $p = 0.010$ ).

Given the heterogeneous size of the lesions, we also examined the relationship between lesion size and number of trials needed to achieve discriminations in the various tasks. Figure 5 illustrates that there was no relationship between lesion size and performance in the discrimination tasks involving canary song and reversal learning. Interestingly though, in tasks 2 and 4, lesion size and performance





**Figure 5** Relationship between discrimination performance (y axis) and lesion size (x axis) in the five consecutive tasks (A–E). The dashed lines show the interaction between lesion size and discrimination performance when

were notably correlated. This is true regardless which lesion location is plotted: Area X alone, IMAN alone, or the average of both. Moreover, including the intact animals as data points with 0% lesion results in strikingly similar slopes, implying that the lesioned animals' performance does predict the performance of the intact animals.

## DISCUSSION

Most natural communication systems have to do with the exchange of signals between members of the same species. The nature of the signal is often instructive in that it tells the recipient what to do, or not to do. We trained birds to perform a task that required them to associate one of two songs with access to food, and to respond accordingly. Stripped to its essentials, the task required our birds to learn that sounds broadcast by the speaker in their cage were a command, and they had to memorize the two sound stimuli and remember which response had to be given to each of them. In the present report, “discrimination” refers to a learned pairing of signal and response.

Although this type of association does not occur in nature, performance on tasks 1 and 5, in which the birds discriminated between different canary songs, shows that an intact Area X and IMAN were not necessary to master any of these steps. However, adult male zebra finches that received lesions of Area X and IMAN required more trials than intact finches to discriminate between their own song and that of a cagemate. These same lesions also affected their ability to discriminate between two novel conspecific songs, although this effect did not reach significance. An examination of the relation between lesion size and discrimination performance (number of trials to reach criterion) across individuals showed a high correlation only for these two

only the lesioned birds are plotted ( $n = 8$ ). The solid lines show the relationship when intact animals are also included as lesion size = 0% ( $n = 16$ ). Bigger square symbols indicate coincident data points. Note the similarity of slopes of both lines in all tasks. Tasks 2 and 4 are the only tasks in which lesion size predicts performance. If intacts are included,  $p$  for both tasks  $< 0.01$ . Lesion sizes plotted are the average of percent Area X and percent IMAN lesioned. However, the findings were equivalent when only Area X or only IMAN were plotted (not shown).

tasks. Taken together, our results suggest that Area X and IMAN (one, the other, both, or closely apposed tissue) play a role not just in song acquisition, as reported earlier (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991), but also in adult conspecific song discrimination.

There are other reports that suggest that the anterior forebrain pathway is involved with the use of learned vocal signals for communication among conspecific adults. For example, DeVoogd et al. (1996) showed that the volume of IMAN in the females of different European warbler species (*Sylviidae*) varies with the number of songs produced by conspecific males. In cowbird females (*Molothrus ater*), which do not sing, the size of IMAN in females is related to their ability to discriminate between different conspecific songs (Hamilton et al., 1997). These observations are congruent with a report that lesions of IMAN in adult female canaries interfere with song discrimination (Burt et al., 1997).

Other observations do not fit as well. Area X and IMAN neurons of adult, awake, male zebra finches do not respond consistently to playbacks of conspecific song (Doupe and Solis, 1997). Moreover, whereas playbacks of conspecific song induce an up-regulation in the expression of some immediate-early genes in various auditory relays of adult, awake zebra finches, such up-regulation is absent in Area X and IMAN (Mello et al., 1992; Mello and Clayton, 1994; Jarvis and Nottebohm, 1997), which would be in line with these nuclei being unresponsive to playback. Intriguingly, electrophysiological recordings from Area X and IMAN in zebra finch males under anesthesia show that neurons in these nuclei respond selectively to presentations of the BOS (Doupe, 1997). If auditory stimuli are not processed by the AF pathway in adult awake animals, then perhaps the difficulty that birds with AF lesions show when trying to master conspecific song discriminations has to do with other (e.g., attentional or memory) aspects of these tasks.

The broadest interpretation of the work of others as well as of our own is that Area X, IMAN, and/or adjacent tissue are necessary for the flexibility needed to acquire, use, and respond to learned vocal signals. In line with this view, lesions of IMAN in juvenile zebra finches impose on these birds a marked song stereotypy that precludes further vocal learning (Scharff and Nottebohm, 1991). The same lesions in adult canaries force an abrupt change from variable to stereotyped song, with a subsequent increase in the monotony of the song produced (Nottebohm et al., 1990; see also Benton et al.,

1997). An intact IMAN is also necessary for adult male zebra finches to correct song changes brought about by syringeal denervation (Williams and Mehta, 1995). Likewise, whereas the song of deaf adult male zebra finches deteriorates over a period of weeks (Nordeen and Nordeen, 1993), IMAN lesions prevent this deterioration (Brainard and Doupe, 1997). Apparently, the absence of IMAN limits plasticity, so neither learning nor forgetting can occur.

Birds in nature may not be able to afford the luxury of training hundreds of times until they master a discrimination. Our observations suggest that Area X and IMAN—or tissue closely apposed to these two nuclei—of adult male zebra finches are necessary for the prompt memorization of the sounds of other conspecifics and/or that these structures are involved in regulating the responses to such sounds. These roles could be an extension of the involvement of these nuclei with processes of vocal learning in juveniles.

Based on connectivity patterns, topographical organization, and developmental and neurotransmitter profiles, the argument has been made that the basal ganglia in mammals and birds have strikingly similar functional circuitry (Medina and Reiner, 1995; Veenman et al., 1997). The AF pathway of songbirds shares many of these similarities (Bottjer and Johnson, 1997). The basal ganglia are thought to be involved with the selection and planning of motor behaviors and higher-order cognitive function (Hassler, 1978; Hikosaka, 1991; Graybiel, 1995). Our findings seem to fit this functional profile. The challenge is now to show what each nucleus of the AF pathway contributes to processes of vocal communication.

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