Hemispheric differences in avian song discrimination

(lateralization/zebra finch/operant/harmonic)

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ABSTRACT   Auditory input to the right or left forebrain of adult male zebra finches (*Taeniopygia guttata*) was disrupted by lesioning the ipsilateral auditory relay nucleus of the thalamus. These birds were then presented with two kinds of auditory discriminations: (*i*) between their own song and the song of a cage mate; (*ii*) between two versions of an unfamiliar zebra finch song that differed only in the harmonic profile of one of the syllables. Right-side lesion birds did better than left-side lesion ones at discriminating between their own song and the song of a cage mate; left-side lesion birds did better on the harmonic profile task. We suggest that the two halves of the zebra finch brain process conspecific sounds differently, as seems to be the case for humans.

Lateralization is one of the more intriguing aspects of brain function. Hemispheric specialization for perception, especially for perception of conspecific vocal signals, has been documented in humans (1–4), macaques (5), and mice (6). Our interest in possible right–left asymmetries in brain pathways for avian song perception stems from the observation of laterality for the production of learned song (7, 8), which is reminiscent of the hemispheric dominance for speech skills observed in humans (9, 10).

We employed a combination of behavioral, surgical, and electrophysiological techniques to test for hemispheric dominance in avian song perception. Birds were given unilateral lesions that disrupted auditory input either to the right or left hemisphere of the song control system (11, 12). They then were tested on two song discrimination tasks that we had employed previously for assessing how intact zebra finches discriminate between conspecific song structures (13, 14). In the first task, the birds had to learn to discriminate their own song from a cage mate's song. In the second task, they had to discriminate between two versions of an unfamiliar conspecific song that differed only in the harmonic structure of one part of the song. This behavioral design was similar to that used by Petersen *et al.* (5) to show hemispheric differences in the perception of conspecific vocalizations by macaques (*Macaca fuscata*).

At the end of behavioral testing, we used electrophysiological methods to document the persistence of unilateral disruption of auditory input received by either hemisphere. We then confirmed the unilateral lesions histologically.

METHODS

Subjects. Fourteen adult male zebra finches (*Taeniopygia guttata*) were divided into seven pairs. Both members of a pair were housed in the same cage but were separated by a wire partition. Water and grit were available to the birds at all times. Full-spectrum fluorescent lights in the aviary paralleled the natural photoperiod for New York state. Four pairs

of birds were tested in June–July (14 hr of daylight) and the others were tested in December–January (9 hr of daylight).

Placement of Lesions in Right and Left Nucleus Ovoidalis. The right or left nucleus ovoidalis [thalamic auditory relay nucleus (11, 12)] of each of these birds was located by recording from glass-insulated tungsten electrodes while playing tones. Birds were anesthetized with a ketamine/xylazine mixture (25 mg and 50 mg per kg of body weight, respectively) and placed in a Kopf stereotaxic apparatus with hollow, perforated earbars and a bill clamp. Tone bursts (1.1–2.2 kHz, 50–100 ms) were played to the birds through a Nagra speaker placed 35 cm from its head. Electrodes (15) were lowered to the approximate location of nucleus ovoidalis using stereotaxic coordinates derived from a canary atlas (16). The boundaries of nucleus ovoidalis were mapped by determining where units fired in response to auditory stimuli.

Of the two birds in each pair, one received a lesion in the left nucleus ovoidalis, and the other bird received a comparable lesion in the right nucleus ovoidalis. Nucleus ovoidalis is partially surrounded by major ascending and descending fiber tracts. Our lesions were conservative to avoid damaging these tracts. Using the map generated through auditory stimulation we aimed our lesions at the ventral border of nucleus ovoidalis, where the ascending fibers from the mesencephalic auditory nucleus arrive (12). Lesions were made incrementally by passing constant current through the recording electrode (maximum: 120 s at 25 μA) and monitoring the auditory responses in field L subsequent to the lesion. Field L, in caudal neostriatum, is the primary target of nucleus ovoidalis and is thus analogous to mammalian auditory cortex (11, 12). A lesion was considered satisfactory when it markedly reduced field L responses to sound. Songs were recorded before and after lesioning to ensure that vocal control pathways coursing near ovoidalis had not been damaged.

Behavioral Stimuli and Procedures. Each bird was then trained to discriminate between its own song and the song of the other member of its pair. A companion paper provides more complete details on this method (13). The two stimuli for each pair consisted of two single, stereotyped courtship songs (17, 18) of approximately the same duration and containing a variety of syllable types (Fig. 1 *A* and *B*). These songs were recorded before the lesions to nucleus ovoidalis were produced.

Latency between surgery and the beginning of training was the same for both members of a pair and ranged between 10 and 100 days. The birds were trained on a *go/no-go* procedure in which perch hopping was the operant response. A bird's own song was always the *go* stimulus while the other member of the pair's song was the *no-go* stimulus. A correct *go* response was rewarded with seeds; an incorrect *go* response resulted in the light in the test chamber going out for 15 s. All *no-go* responses resulted in the end of the trial after
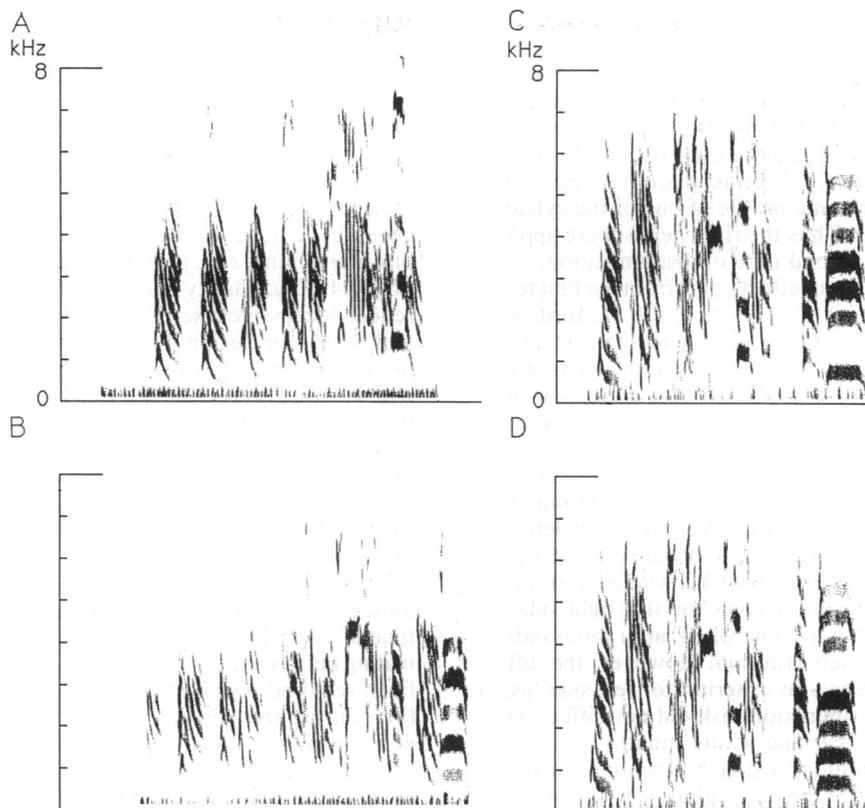
Abbreviation: HVC, high vocal center.

FIG. 1. (*A* and *B*). One of the seven pairs of songs used in the initial discrimination. The *x* axis represents time, with the song in *A* 700 ms in duration. The *y* axis represents a frequency range from 0 to 8 kHz. (*C* and *D*) The two stimuli used in the second discrimination task. They are identical except for the respective final syllables, which differ in harmonic structure. The *go* stimulus (*C*) for all birds had a syllable with an attenuated second harmonic, and the *no-go* stimulus (*D*) had the same syllable with an attenuated fifth harmonic. The two stimuli were edited from the same song, which was not familiar to any of the birds in this experiment. Power spectrum measurements at the position of a bird's head when listening to the stimuli showed that the attenuated harmonics were a minimum of 30 dB below the level of any of the two adjacent harmonics.

3 s. Birds were food-deprived 4–6 waking hr before each session. Daily sessions were 3–4 hr in duration, with both birds in a pair trained simultaneously in separate operant cages.

Once this discrimination had been mastered, the same procedures were used to train the birds to discriminate between two versions of an unfamiliar conspecific song that differed only in the harmonic profile of one of its syllables (Fig. 1 *C* and *D*). Central auditory mechanisms have been implicated in avian recognition of harmonic structures (19), and pitch perception of complex harmonic structures is affected by unilateral focal cerebral lesions in humans (20). Zebra finches can master a discrimination between conspecific song syllables that differ in harmonic structure when the song syllables are either presented in isolation or embedded in song (14, 21).

Electrophysiological Recordings to Determine Lesion Effects in a Song Control Nucleus. At the end of the behavioral experiments, four birds were again anesthetized with a ketamine/xylazine mixture and placed in the stereotaxic apparatus. A single glass-insulated tungsten electrode was used in a total of eight recording sites [two in field L and six in high vocal center (HVC)] for each bird. HVC receives an auditory input from field L (11, 12, 22), is essential for normal song production in passerine birds (7), and also includes neurons with song-specific auditory responses. From the latter observation it has been suggested that HVC plays an important role in song discrimination (23). Auditory stimuli were 50-ms tone sweeps centered on 1.4 kHz. The electrode was placed initially in the right HVC, and the best auditory response along that track was localized and recorded as an average of

responses to 10 consecutive stimuli presented at 3-s intervals. The coordinates for this penetration were then reflected about the midline to establish a recording site in the left HVC, and the procedure was then repeated starting with a new site in the left HVC. Recordings from field L followed the same procedure, but the side recorded first alternated between birds.

Histology. All birds were eventually killed to document the placement of the lesion. Birds received an overdose of anesthetic (either Chloropent or sodium pentobarbital) and were then perfused with 30 ml of 0.9% NaCl followed by 30 ml of 10% formol saline. Sections (50 μm) were cut on a Vibratome and stained with cresyl violet. The size and extent of lesions were determined by visual examination (scarring and microglia were the best markers) to provide anatomical support for the physiological evidence obtained at the time the lesions were made.

DATA ANALYSIS AND RESULTS

Behavior. The acquisition criterion was set at 75% correct, which is halfway between chance and errorless performance. To determine significance, we used a hierarchical repeated measures analysis of variance, with the right- or left-side lesions and the seven pairs of birds as factors across the two discrimination tasks. Planned comparisons were used to analyze simple effects unless otherwise noted. One bird died before the start of the second discrimination task.

The side of the lesion was significant, $F(1, 5) = 23.44$; $P < 0.01$, with the difference between trials to acquisition in the first discrimination task significant at a $P < 0.05$ level. This

indicated that birds with the left-side lesions required more trials than did birds with the right-side lesions in each pair to learn the discrimination between their own song and the song of the other bird (Fig. 2*A*). We infer that the left hemisphere is dominant for this kind of discrimination. In contrast, birds with right-side lesions required more trials than did birds with left-side lesions to learn the discrimination between songs differing only in the harmonic profile of one of the syllables (Fig. 2*B*), $P < 0.05$ level. Thus the right hemisphere appears to be dominant for the second type of discrimination.

There was also a significant effect going from the first to the second discrimination, $F(1, 5) = 65.36$; $P < 0.01$. Birds with the left-side lesions showed no significant change in the number of trials to acquisition in going from the first discrimination to the second, while birds with right-side lesions required significantly more trials to acquire the second discrimination, $P < 0.01$.

We also encountered a seasonal effect, $P < 0.05$, as measured with a post hoc test, for the first discrimination (own song versus cage mate's song). Both right- and left-side lesioned birds learned the first task in fewer trials when the days were long. This test was not possible with all birds in the second discrimination because some of the right-side lesioned birds were halted in training when, after thousands of trials, they had not reached criterion. However, the difference in the number of trials to criterion for left-side lesion birds in the second discrimination task did not differ significantly between the summer and winter months.

Electrophysiology. Auditory evoked potentials recorded from field L and HVC after the behavioral experiments were smaller or absent on the side of ovoidalis lesions in all four birds examined (Fig. 3). These birds had undergone ovoidalis lesions 21 and 24 months before the electrophysiological recordings were made. We take this as confirmation that our lesions disrupted auditory input to the auditory telencephalon and to the song control pathway and that these lesions were not followed by recovery that eliminated the functional asymmetry.

Histology. Twelve of the 14 brains yielded clear evidence of thalamic electrolytic lesions. The other two birds did not yield satisfactory histology. In the birds with good histology, the lesions were in nucleus ovoidalis.

DISCUSSION

The simplest explanation for our results is that the superior left side performance in the first task occurred because the

stimuli were the bird's own song and that of a cage mate, whereas the superior right side performance in the second task resulted from the subtlety of the harmonic differences that had to be recognized and remembered. The order in which the tasks were presented could conceivably have affected the hemispheric dominance observed for each task, but, for the reasons that follow, this seems unlikely.

Zebra finches trained on a series of song discriminations generally require progressively fewer trials to master the discriminations, except in the case of a reversal discrimination (24). Familiarity with the testing situation seems to render successive discriminations easier. The great difficulty that right-side lesion birds encountered with the second discrimination did not follow this pattern. Moreover, birds with left-side lesions did not find the second task harder than the first one. Thus, the increase in difficulty seems to be determined more by the side of the lesion than by the order of the experiments. We do not know whether song processing by the intact side involved both field L and HVC, but we suspect, from reports in the literature (23), that this may have been the case.

A previous experiment using intact birds showed that difficulty in song discrimination can also be affected by the time of year (13). In that experiment, zebra finches discriminating between their own song and the song of another zebra finch required fewer trials to reach criterion during summer than during winter. We now have observed the same phenomenon in unilaterally lesioned birds. However, the seasonal effect may not extend to other discriminations since, for example, there was no evidence of a seasonal effect for the second task in the present report. Elsewhere, we suggest a possible mechanism for the seasonal effect (24).

Although we do not know what factors determined the relative ease of the two discriminations or the task-specific hemispheric dominance, the observation of functional asymmetry is real, even if the mechanisms remain unknown.

Hemispheric asymmetry for speech and language skills affects both perception and production (1–4, 9, 10). If vocal communication in birds that learn their song is governed by principles of brain function similar to those found in humans, then in birds, too, production and perception of learned vocal signals may be represented unequally in the two hemispheres. Left-side dominance for song production has been shown in canaries (8, 25). Although the picture is not as clear in zebra finches, their right side may be dominant for song production (26). The present results suggest that the two hemispheres of songbirds perceive and process conspecific
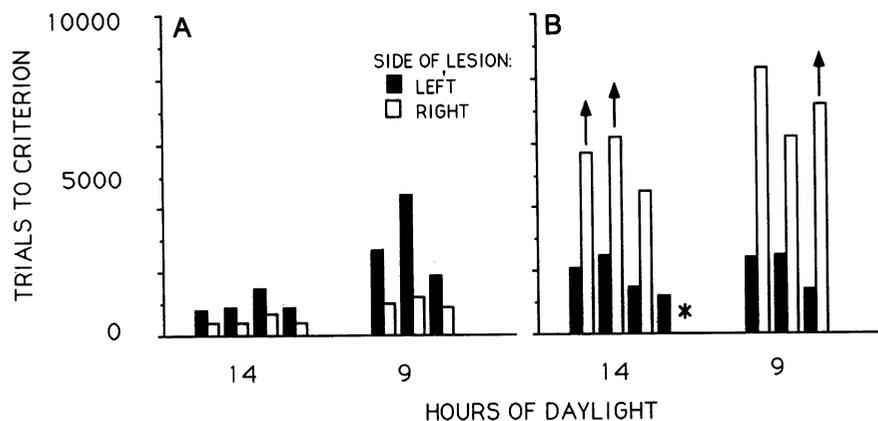

FIG. 2. (*A*) Trials to learning criterion for each zebra finch when trained to discriminate between its own song and the song of a cage mate (see stimulus examples in Fig. 1 *A* and *B*). In each of the seven pairs, a bird received a lesion to either the left or the right nucleus ovoidalis. The numbers along the *x* axis indicate the amount of daylight for the summer and winter experimental series. (*B*) Trials to criterion for the same birds when subsequently trained to discriminate between two versions of an unfamiliar song that differed only in the harmonic profile of one syllable (Fig. 1 *C* and *D*). Testing of right-side lesion birds in pairs 1, 2, and 7 in the second task (represented by arrows above bars) stopped before the birds reached learning criterion. One bird, indicated by an asterisk (pair 4), died before the second experiment began.
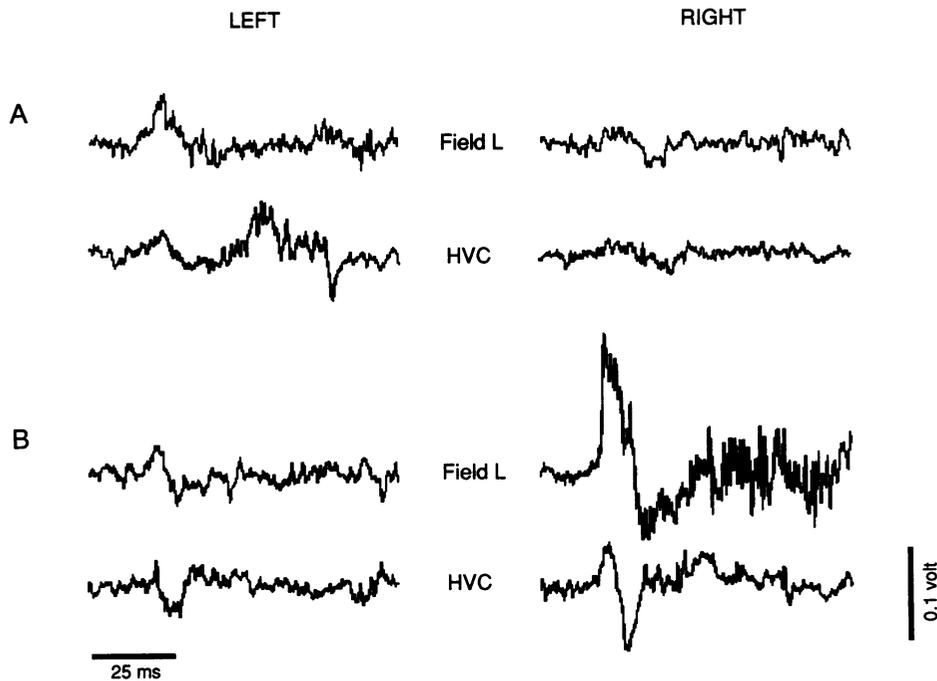
FIG. 3. Lesions in nucleus ovoidalis disrupted auditory responses in the ipsilateral field L (its primary auditory projection) and HVC (a song control nucleus), as illustrated by responses with two birds (*A* and *B*). Comparisons should be made between the right and left sides of the same bird, which were recorded with the same electrode. Lesions did not destroy the entire nucleus (for fear of damaging the major adjacent fiber tracts) but reduced the amplitude of ipsilateral auditory responses even after more than 1 year had passed. Lesioning the right ovoidalis (*A*) diminished the evoked auditory potential in the ipsilateral field L and HVC with respect to the unoperated side. Lesioning the left ovoidalis (*B*) decreased the amplitude of the evoked auditory potential in the ipsilateral field L and HVC, while the right side continued to respond vigorously. Each pair of traces (left versus right) was matched by stereotaxic coordinates and is the average of 10 stimulus presentations. The stimulus, a 50-ms tone upsweep (1–2 kHz), began 10 ms after the recorded trace onset.

vocal signals differently. Our observations add to the many parallels between the two systems—avian and human—that have become paradigmatic of vocal learning (27, 28).

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