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Effect of Tracheosyringeal Nerve Section on Sexually Dimorphic Distance Calls in Bengalese Finches (*Lonchura striata var. domestica*)

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ABSTRACT—In Bengalese finches, adult male distance calls are narrow-banded in the spectral domain and continuous in the time domain while female calls are wide-banded and pulsed. To examine the degree of syringeal control on this sexually dimorphic behavior, the nerve bundle from the hypoglossal nucleus to the syrinx was cut unilaterally in adult birds under deep Nembutal anesthesia. The sexual dimorphism in the time-amplitude profile of the distance calls did not disappear by cutting neither the left nor the right nerve. On the other hand, the sex difference in the spectral domain of the distance call became more ambiguous by cutting the left nerve but only minor effect was observed by cutting the right nerve. Results suggest that only spectral information is conveyed by the tracheosyringeal nerves and calls of the both sexes are actively controlled by the left tracheosyringeal nerve. Some of these results are in contrast with the zebra finch whose right tracheosyringeal nerve is dominant over the left and the female call does not change by the nerve cut operation.

INTRODUCTION

Bird vocalizations provide a prominent model to the study of the sexual differentiation of behavior [1]. Among bird vocalizations, song is usually present only in the male but calls are shared by the both sexes [2]. Consequently, although the study of song can provide correlations as to the existence of some neural substrate and the existence of some behavior, analysis of calls have possibility to provide insights on how certain class of behavior could be expressed differently in the male and female.

Some of the most common calls emitted by songbirds are distance calls. These calls are emitted to confirm each other's location when a bird lost sight of its mate or company [18]. Distance calls are sexually dimorphic in the zebra finch (*Taeniopygia guttata*) [19]. The constant frequency portion of male calls are shorter and higher-pitched than that of female calls. In addition to the constant frequency part, male calls have an auxiliary element which consists of rapid frequency modulation [6, 18]. These masculine properties of the male distance call are learned during development [19]. When deprived of the opportunity to learn from conspecific males, zebra finch males develop distance calls with many of the masculine properties missing [6, 10, 19].

Bird vocalization is controlled by respiratory activity and syringeal muscles [13]. The syringeal muscles are innervated by the tracheosyringeal branch of the hypoglossal nerves (NXIIts) which extend bilaterally from the syringeal part of the hypoglossal motor nucleus (nXIIts). In zebra finches, the masculine properties except for the shorter duration of the male distance calls disappeared after cutting NXIIts bilaterally but female distance calls remained unchanged with the same operation [10, 15]. The same effects could be obtained by cutting the right NXIIts only [14].

The data available on the zebra finch distance calls suggest the following 3 points. First, distance calls are actively controlled by the NXIIts in the male but not in the female. Second, sexually dimorphic features except for the duration are conveyed by the NXIIts. And finally, the right NXIIts is dominant over the left in controlling the sexually dimorphic features in zebra finch distance calls. In the present paper, we examined the generality of these findings by cutting unilateral NXIIts in the Bengalese finch (*Lonchura striata var. domestica*).

The Bengalese finch belongs to the same family as the zebra finch: both are Estrildid finches of the family Ploceidae [4]. The most prominent features of the sex differences in the Bengalese finch distance calls can be summarized in 3 parameters. Distance calls are wide-banded, low pitched and pulsed in females and narrow-banded, high pitched and continuous in males [7, 17]. The first 2 parameters can be considered as "spectral" parameters while the last parameter is a "temporal" parameter. We were interested in how these sexually dimorphic parameters would change by cutting the NXIIts nerves.

MATERIALS AND METHODS

Bengalese Finches and their maintenance

Birds were obtained from a local pet supplier and kept in an aviary under a fixed 14-hr-light/10-hr-dark cycle. Ambient temperature of the aviary was maintained around 24°C and relative humidity was around 60%. Birds were kept in a small cage, each housed 3–4

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birds. A total of fourteen birds (8 males and 6 females) were used in this experiment. The tracheosyringeal branches of the hypoglossal nerves (NXIIts) were cut bilaterally in one male bird. In another male bird, a bleeding complication occurred during the surgery which made it difficult to cut the NXIIts. Consequently, this bird was used as a control bird. The remaining 12 birds were randomly divided into a left-cut group and a right-cut group, both groups containing 3 males and 3 females.

Surgery

We followed the basic surgical procedure described in Williams and McKibben [15]. Briefly, the bird was anesthetized by Nembutal (0.012 ml/bird) and feathers were pulled out from the neck. Local anesthesia (Xylocaine) was applied on the skin and an incision was made to expose the trachea. A pair of fine forceps was used to grab the tracheosyringeal nerve. The nerve was pulled and 5–10 mm portion of it was cut. The incision was sutured and anti-biotic was applied. The bird recovered from anesthesia within 3 hours after the surgery. The sign of nerve re-growth was examined after the final recording was made. In no case nerve regrowth was observed.

Recording

Calls were recorded in a quiet sound proof room. A cage containing 3–4 birds were brought into the room and one of the birds was moved into another cage. By visually isolating this bird from the others, we were able to elicit distance calls from the bird [17]. An electrolet condenser microphone (AIWA CM-51) connected to a digital audio tape recorder (AIWA HD-S1) was used to record the calls. Distance calls were first recorded at least one month prior to the surgery. The second recording took place 1–3 days before the surgery. These two recordings were compared to make sure that the call was stabilized and no further change due to development occurred [17]. Post operative recordings were first made within 24 hr of the surgery. Recordings were made occasionally until 16 weeks after the surgery.

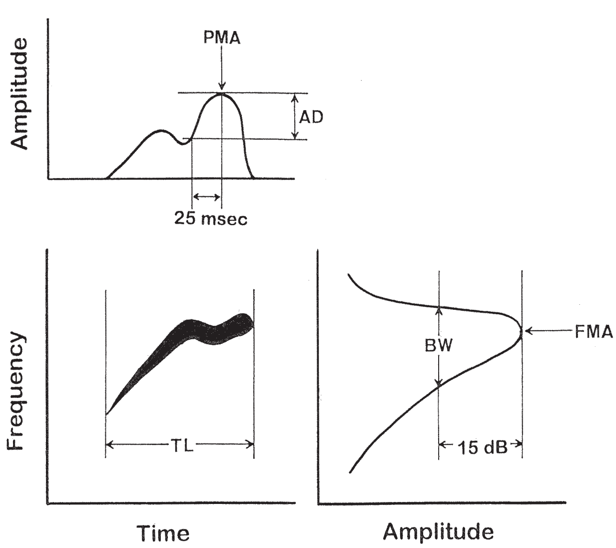

Fig. 1. Three parameters taken on the distance call are explained. The upper figure is the amplitude profile of a model distance call. The lower left figure is the soundspectrogram of the call and the lower right figure is the total power spectra of the call. Abbreviations: PMA = place of the maximum amplitude, AD = amplitude difference, TL = total length, FMA = frequency of the maximum amplitude, BW = bandwidth.

Analyses

Sonograms (soundspectrograms) of the calls were obtained by a digital signal processor (KAY Sona-Graph 5500) at a sampling rate of 26 kHz, analysis bandwidth of 300 Hz. The time course of post-operative changes was examined on these sonograms.

From each of the recordings made just prior to the surgery and 2 weeks after the surgery, 5 most intense calls were selected for further analysis for each individual bird. A soundspectrogram, total power spectrum, and a time-amplitude plot were obtained by a 150 Hz analysis band for each call by the signal processor. The following 3 parameters were taken from these analyses. We previously showed that the parameters similar to these were effective in characterizing sexual dimorphism in Bengalese finches distance calls [7, 17]. Figure 1 explains these parameters in schematic fashion.

1) Frequency of maximum amplitude (FMA). This value was obtained from the total power spectrum of the call.

2) Bandwidth (BW). This value was also obtained from the total power spectrum of the call. The range of frequency occupied by the call at the energy level 15 dB below FMA was defined as BW of the call.

3) Amplitude Difference (AD). The place where maximum amplitude (PMA) occurred in the call was first determined on the amplitude profile. From this point, amplitude profile was traced back for 25 msec. The minimum amplitude within this 25 msec interval was expressed with the level of the place where maximum amplitude occurred as a reference.

FMA and BW dealt with spectral aspects of the call. AD coded temporal dynamics of the call. The sex differences were tested by Mann-Whitney *U* for each of the parameters for pre-operative calls using each individual's average values for the parameters. The sex differences were also tested for post-operative calls for the parameters which showed sex differences in the pre-operative test. Since the number of subjects in each group was only 3, the exact probability was calculated for these analysis. The effect of nerve cut was examined in each individual by Mann-Whitney *U* test for each of the parameters. Effects were further examined on each of the left-cut and the right-cut group by Wilcoxon's matched pair test. Alpha equals to or smaller than 0.05 was required for significance.

RESULTS

Pre- Operative Sex Differences

Before the surgery, distance calls of the males and females were significantly different in 2 parameters out of the 3 measured; BW was wider and AD was larger in females than in males. Although FMA was higher in males, the difference was not significant in the present samples. These results were summarized in Table 1. Sex differences in pre-operative parameters confirmed our earlier findings (for AD, see [17]; for BW, see [7]).

Effects of Surgery

Distance calls recorded just before and two weeks after the surgery were compared in Figure 2 for 5 representative cases. Morphology of the calls changed radically after the surgery when the left side of the nerve was cut but not as much when the right side of the nerve was cut in both sexes. After one week from the surgery, the shape of the call

TABLE 1. Pre-operative sex differences

Subject		AD(dB)	FMA(Hz)	BW(Hz)
Male	Avg	3.2	4168	1158
(n=8)	Sd	1.9	510	233
Female	Avg	13.8	3875	1971
(n=6)	Sd	4.1	440	562
U (df=1)		0	31	0
P*		.002	.366	.002

*, P is an exact probability.

seemed to be stabilized. From that point the call did not change until the final recording which was made 16 weeks after the surgery. In all cases, cutting right nerve only slightly changed the morphology of the calls but that changed radically by cutting the left or both nerves. Thus in Bengalese finches, the left hypoglossus appears to be dominant over the right side.

Post- Operative Sex Differences

For the right-cut group, the significant sex differences in AD and BW were retained. However, the sex differences disappeared in BW for the left-cut birds (Table 2 and Fig. 3). Still, male calls and female calls are clearly distinguishable by examining the sonograms (Fig. 2). This is largely due to the fact that sex difference in AD was preserved even after the left-cut operation and AD was the most prominent sex difference observed in Bengalese finch distance calls (Fig. 4).

Analysis of Individual Cases

Dramatic changes were observed in the bandwidth (BW). With the six birds in each group combined, BW of the calls became slightly, but significantly narrower after

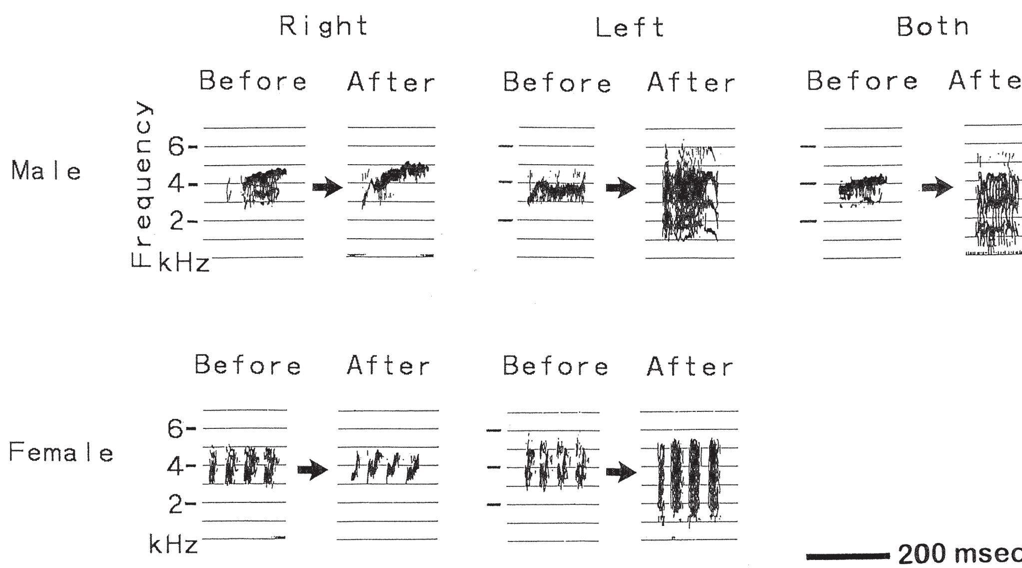

FIG. 2. Examples of distance calls recorded before and after the surgery. Note that the bandwidth increased after the left nerve was cut but decreased after the right nerve was cut in both males and females.

TABLE 2. Post- operative sex differences

Side of operation	Subject	AD(dB)	BW(Hz)
Right	Male	3.5	1088
	Female	18.8	1501
	P*	.05	.05
Left	Male	3.9	3353
	Female	13.2	4454
	P*	.05	.51

For each group n=3. *, P indicates an exact probability.

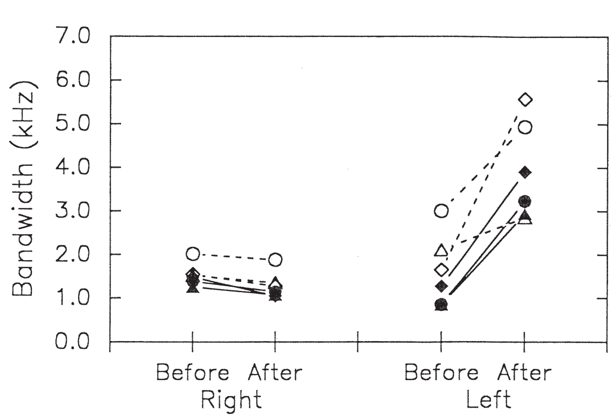

FIG. 3. Changes in the bandwidth of the calls before and after the surgery. Filled symbols=male calls, open symbols=female calls.

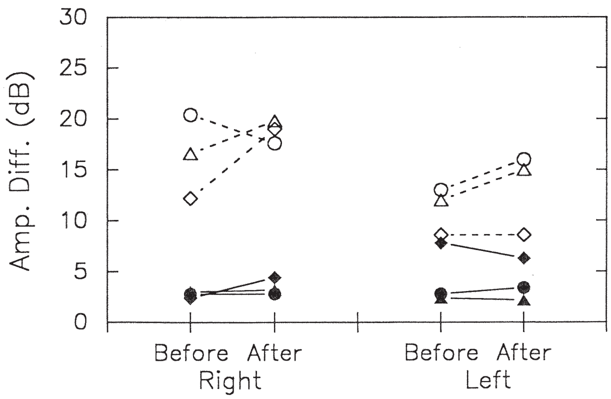

Fig. 4. Changes in the amplitude difference of the calls before and after the surgery. Filled symbols=male calls, open symbols=female calls.

Table 3. Individual summary

Side of operation	Subject: Bird	Subject: Sex	Variables: AD(dB)	Variables: BW(Hz)
Right	GX	F	ns	D*
	XW	F	ns	ns
	GBX	F	I**	D*
	WR	M	ns	D**
	RY	M	ns	ns
	GR2	M	ns	D**
Left	YX	F	ns	I*
	XB	F	I*	I*
	XY	F	ns	I**
	WW	M	ns	I**
	RG	M	ns	I**
	RR	M	ns	I*
Bilateral	BY	M	I*	I*
control	GR	M	ns	ns

Abbreviations: F=female, M=male, ns=not significant, D=decrease, I=increase, *=$P<0.05$, **=$P<0.01$

cutting the right nerve ($P<0.05$) and BW got much wider by cutting the left nerve ($P<0.05$; Fig. 3). On the other hand, temporal parameters did not show such dramatic changes. On average, amplitude difference (AD) did not change by neither the left nor the right cut operation (Fig. 4).

Table 3 summarizes each individual case for the change in each parameter before and after the surgery. Not surprisingly, the largest change occurred when both nerves were cut (BY) and no change occurred in the control bird (GR).

DISCUSSION

Sex differences in the temporal domain of distance calls in Bengalese finches did not disappear by sectioning the NXIIts. But the nerve cut operations had effects on the spectral domain of the calls. This finding is in parallel with that reported on the zebra finch [10]. But unlike zebra finches in which the effect of the nerve cut appeared only in male calls but not in female calls [10], not only male calls but also female calls were influenced by the surgery. This implies that in Bengalese finches, distance calls of both sexes are actively controlled by the tracheosyringeal nerves.

Left-side dominance

Severing the left or right side of NXIIts had different effects on the morphology of distance calls in Bengalese finches. Cutting the left nerve changed calls of the both sexes into noisy, unpleasant calls but the operation on the right nerve did not change the feel of the calls. Thus it is apparent that the left NXIIts is dominant over the right NXIIts in Bengalese finches. When parametric analyses were conducted, however, the effect of cutting the right nerve did appear; bandwidth of the call decreased slightly by cutting the right nerve, while that increased largely by cutting the left nerve.

Among estrildine finches, the side of dominance for vocal control had been examined in the zebra finch [14] and in the Java sparrow [9]. Both the Bengalese finch and the Java sparrow are "left-handed" while the zebra finch is "right-handed". Williams et al. suggested that the side of dominance for vocal control might be a useful taxonomic tool [14]. This might indeed be the case. The Java sparrow and the Bengalese finch are both Asian estrildine finches but the zebra finch is an Australian finch. Examining another estrildine species of Australian origin would be interesting.

Pathways for temporal and spectral information

The nerve cut operations did not change the amplitude profile, one of the most prominent sex differences in Bengalese finch calls. But, since we did not examine the effect of bilateral nerve section in female birds, there is a room to argue that the amplitude profile in female distance calls might be modulated by cooperative action of the left and right syringeal nerves. If it is the case, bilateral sectioning of these nerves might indeed change the amplitude profile. However, we do not think this is the case. Recent studies by Suthers [11] indicates that sound output is a result of a linear summation of the left and right syringeal activities. In the present study, the amplitude characteristic of the female distance call did not change by cutting either side of the tracheosyringeal nerves. Thus it is unlikely that cutting both sides could change the amplitude profile.

Of course, there still is a possibility that the sex differences in amplitude profile of the call might be due to peripheral differences. Again, we do not think this is the case since there is a period of coexistence of male-type calls and female-type calls in male Bengalese finches [17].

If it is not due to the peripheral differences, respiratory output coordinated with syringeal function must be responsible for the sex differences in the amplitude profile of the calls. Recent anatomical study [12, 13] on the zebra finch brain suggested that after the level of the telencephalon, respiratory control and syringeal control were conveyed via different pathways. The output nucleus of the archistriatum (RA), after projection to the dorsomedial nucleus of midbrain intercolliculalis (DM), projects to three targets: the syringeal part of the hypoglossal motor nucleus (nXIIts), nucleus ambiguus, and rostral ventrolateral medulla. The nucleus ambiguus projects to larynx and, via retro ambiguus, to expiratory motoneurons [13].

Since in the present study cutting the NXIIts did not change amplitude profile of the calls, which is presumably controlled by respiratory activity, the present behavioral data fit well with the anatomical knowledge. Laryngeal and respiratory outputs must be conveying the temporal information of distance calls.

Learning and brain pathways

In zebra finches, masculine properties of distance calls are learned [19] and learned components of the call are controlled via NXIIts [10]. Females calls are not learned and cutting NXIIts does not affect them [10]. From these data, Simpson and Vicario suggested that "brain pathways for learned and unlearned vocalizations differed in zebra finches". As to Bengalese finches, we at least know that learning is not involved in the expression of the female distance calls since early deafening does not interfere the normal development of female calls [16]. Our results suggest that the active control of syringeal muscle is necessary in producing female distance calls although it is not learned. Thus in Bengalese finches, learned and unlearned vocalizations probably utilize the same brain pathways, at least after the level of the telencephalon. From the comparison of these two species, we can infer that the involvement of the nXIIts is independent from whether the call is learned or not.

Conclusion

The zebra finch has been a "white rat" in the study of the behavioral and anatomical correlates of birdsong [3]. Comparative work on a closely related species would be important in confirming and extending the findings obtained in one species [5]. The side of dominance for vocal control has been extensively studied in the zebra finch [8, 10, 14, 15] and our data on the Bengalese finch vocalization should provide such comparisons.

In extending the generality of the zebra finch data, since the sex difference in the amplitude profile did not change after the nerve cut operation, we were able to supply a support for the idea raised though anatomical data that temporal and spectral pathway may be different [12, 13].

In emphasizing the species differences, we show, unlike the zebra finch, the left side is dominant over the right in the Bengalese finch. We also show, unlike the zebra finch, female calls which are not learned still utilize the NXIIts in the Bengalese finch.

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