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Authors: Fukushima, Yasuhiro, and Aoki, Kiyoshi

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# Neural function of the mesencephalic dorsomedial nucleus (DM) on distance call production in Bengalese finches

Yasuhiro Fukushima, Kiyoshi Aoki\*

*Sophia University, Life Science Institute 7-1 Kioi-cho,  
Chiyoda-ku, Tokyo, Japan*

**ABSTRACT**—In sexually mature Bengalese finches, acoustic structures of distance calls show sexual difference. The dorsomedial nucleus (DM) of intercollicular complex is known as the midbrain vocal center of distance calls. Neural input from the robust nucleus of archistriatum (RA) was observed in the DM of sexually mature males, but not observed in that of sexually mature females. The purpose of this study is to clarify some more details of physiological function of the neural system in the DM in distance call production. Electrical stimulation to the DM of both sexes induced a call acoustically similar to distance calls, whose duration depended on the number of the pulses/train of electrical stimulation; electrical stimulation in relatively large (or small) numbers of pulses/train induced calls with relatively long (or short) duration, respectively. Multi-unit spikes were recorded from neurons in the DM. The increment of the frequency of recorded spikes was large when the bird vocalized distance calls, and the number of the frequency decreased when the bird vocalized calls whose duration was shorter than that of distance calls. These results suggest that the neural system in the DM controls duration of distance calls in sexually mature males and females. Electrical stimulation to the DM under different pulse frequencies induced calls with different patterns of time-frequency characteristics. The relation between the pulse frequencies and time-frequency characteristics showed sexual difference. The relation between them in RA-lesioned males was similar to that in females. These results suggest that the neural circuit in the DM of sexually mature males is consisted of sexually common neural circuit controlled by the neural input from the RA, and that these sexually different neural system produce sexually different acoustic structures of distance calls.

**Key words:** neuroethology, Bengalese finch, distance call, sexual difference, call production system

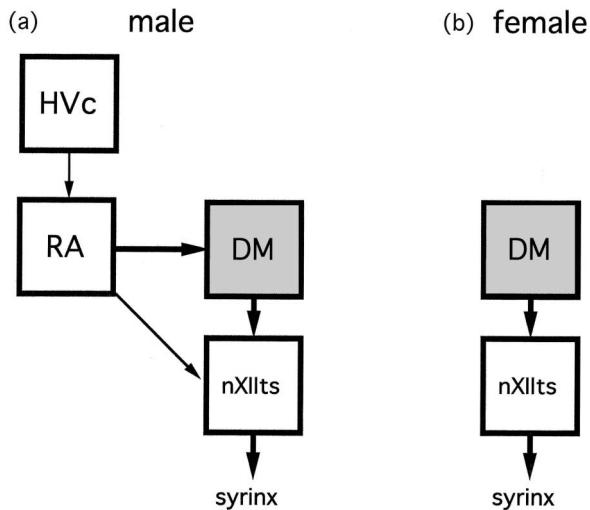
## INTRODUCTION

In passerine birds, males and females vocalize distance calls, when the isolated bird hears the call of its conspecific (Zann, 1985). In sexually mature Bengalese finches (*Lonchura striata* var. *domestica*), the acoustic structures of such distance calls show sexual differences (Okanoya and Kimura, 1993). Males and females sometimes vocalize short calls, which have sexually similar acoustic structures and a shorter duration than the distance calls. In the central vocal control system, the dorsomedial nucleus (DM) of the intercollicular complex in the midbrain is known to be one of the motor centers of call vocalization in males and females (Seller, 1981), because electrical stimulation to the DM in some species of passerine birds induces vocalization acoustically similar to calls (Brown, 1965a; Vicario and Sim-

pson, 1995; Fukushima and Aoki, 2000), and bilateral lesions of the DM eliminates calls (Brown, 1965b; Fukushima and Aoki, 2000). In male Bengalese finches, electrical stimulation to the DM did not induced vocalization acoustically similar to song element, and electrical stimulation did not elicit song (Fukushima and Aoki, 2000). As shown in Fig. 1, the DM send axons to the tracheosyringeal nucleus (nXIIts) in male and female Bengalese finches. The nXIIts innervates muscles of the syrinx. However, the neurophysiological function of the DM in the vocalization of distance calls is almost unknown.

Sexually mature male Bengalese finches sing songs with complex acoustic structures and long duration, but females do not. Corresponding to this sexual dimorphism in singing behavior, the motor control system of song vocalization resides in the brain in males, but not in females. In males, the motor control system of song vocalization is developed during maturation (Nottebohm and Arnold, 1976). This system includes the hyperstriatum, ventrale caudalis (HVc), the robust nucleus of archistriatum (RA) in the fore-

\* Corresponding author: Tel. +81-3-3238-3490;  
FAX. +81-3-3238-3490.  
E-mail: k-aoki@hoffman.cc.sophia.ac.jp



**Fig. 1.** The vocal control system of distance call in males (a) and females (b). This diagram shows most of the known nuclei and connections of vocal control system. All arrows indicate anterograde connections. Neural signals for call descend the pathway to the syrinx via the RA, the DM and nXIIIts in that order as shown by the thick arrows. Abbreviations: HVc, the hyperstriatum, ventrale caudalis; RA, robust nucleus of archistriatum; DM, dorsomedial nucleus of the intercollicular complex; nXIIIts, the nucleus nervi hypoglossi, the tracheosyringalis nucleus

brain, the DM in midbrain, and the nXIIIts in the hindbrain (Nottebohm *et al.*, 1976). In the pathway, neural connection exists from the HVc to the RA, from the RA to both the DM and the nXIIIts, and from the DM to the nXIIIts (Fig. 1 (a)).

The RA is related to production of the male-specific acoustic structure of the distance calls, because bilateral RA-lesioned males vocalize distance calls acoustically similar to those of females (Simpson and Vicario, 1990; Fukushima and Aoki, 2000). To explain the neural mechanism which produces sexually dimorphic acoustic structures of distance calls, Simpson and Vicario (1990) from the results using zebra finches hypothesized two neural pathways in relation to distance call vocalization with sexually different acoustic structures, the “learned” and the “unlearned” pathway. The “learned” pathway in males is the pathway from the RA directly to nXIIIts, and the pathway is thought to control the male-specific element of time-frequency characteristics in distance calls. The “unlearned” pathway in both sexes is the neural pathway from the DM to the nXIIIts, and the pathway is thought to control sexually common elements (especially call duration). Simpson and Vicario thought that the DM is included in the “unlearned” pathway, and that the neural circuit of the DM shows sexually similar function in distance call vocalization. Simpson and Vicario did not mention the function of the neural pathway from the RA to the DM.

Fukushima and Aoki (2000) showed from neuroanatomical experiments on sexually mature Bengalese finches that neural pathway from the RA was observed in the DM of males and not in the DM of females; moreover, electrical

stimulation to the DM induced calls with sexually different acoustic structures. At least in Bengalese finches, these results could not be explained in simple logic by the hypothesis of Simpson and Vicario (1990). Fukushima and Aoki expanded the hypothesis of Simpson and Vicario, that the neural circuit in the DM have sexually different functions in distance call vocalization and the neural connection from the RA in the DM plays a key role in the vocalization of distance calls in sexually mature males. How the neural connection from the RA contributes to the function of vocal control system in the DM of males, however, was not analyzed by neurophysiological methods. For example, the relation between the patterns of electrical stimulation and acoustic structures of induced calls has not analyzed in details by Vicario and Simpson (1995). Moreover, spike discharges from neurons in the DM of passerine birds corresponding with calling behavior have not been observed.

Our purpose in this study is to identify some more details of neurophysiological function of the DM in producing distance calls with sexually different acoustic structures in sexually mature Bengalese finches. First, in order to clarify the neurophysiological function of the DM, we electrically stimulated the DM under different stimulus conditions. The calls thus induced by electrical stimulation (CIES) were acoustically analyzed in males, males after bilateral RA lesioning, and females. Second, to assess the process of sexually different neural circuit of the DM in vocalizing the distance calls, we recorded spike discharges from neurons in the DM corresponding with distance call vocalization.

## MATERIALS AND METHODS

This study used sexually mature Bengalese finches (28 males, 13 females) purchased from a commercial source and housed in an aviary maintained at 22–28°C in a 14 hr light-10 hr dark cycle.

Distance calls were recorded in a sound-attenuating box (Industrial Acoustic Inc.) with a digital audio tape recorder (TCD-D10, Sony). Acoustic structures of the calls were analyzed with a sound spectrograph (DSP Sona-Graph Model 5500, Kay Electronics Corp.). A call was defined as a vocalization that is induced by playback, and is followed and preceded by at least 50 ms of silence. A call that lasted more than 100 ms was defined as a distance call, and one lasting less than 100 ms was defined as a short call. A note was an element of a call. A note in a call was defined as the continuous sound. A call without any silent parts in it was composed of 1 note. The duration, number of notes, and the highest and lowest frequencies of each distance call were measured on a sound spectrogram. All data in this paper was indicated as mean±S.E.

### Electrical stimulation

Stimulating electrodes were implanted in 19 males and 7 females. After a bird was deeply anesthetized with a mixture of ketamine and xylazine (5 µl/g body weight), it was placed in a stereotaxic instrument and a stereotaxic atlas was used to identify the target nucleus of the DM. The skin in the top of the head was removed, and a window (approximately 1 mm × 1 mm) in the top of the skull over the target brain area was opened. An insect pin electrode (No.00, Shiga; insulated except at the tip 10–30 µm) was then inserted vertically into the target nucleus of the DM, and a reference

electrode (No.00, Shiga; not insulated) was placed on the dura mater. The two electrodes were cemented to the skull with dental resin and acrylic glue. The bird was kept warm, and recovered in 2–3 hr.

Electrical stimulation was administered under freely moving conditions. Several trains (10–30) of anodal pulses (duration 500  $\mu$ s, <150  $\mu$ A) were administered at several frequencies (mainly 70–100 Hz). Calls induced by electrical stimulation (CIES) and timing of the electrical stimulation were recorded on a personal computer (PC-9801VX, NEC) through an oscilloscope (VC-9, Nihon Kohden). After electrical stimulation, electrolytic lesions were made (DC 10  $\mu$ A, 10 s) at the stimulation site. Histological sections of stimulated brain tissue were prepared as in our previous report (Fukushima and Aoki, 2000) and then observed under a microscope.

### RA lesions

A week prior to electrode implantation, bilateral RA lesions were made on 7 of 19 males. The bird was anesthetized, placed in a stereotaxic instrument, and the window of the skull was opened in the same method as for inserting electrodes. An insect pin (Shiga No.00, insulated except at the tip 120–150  $\mu$ m) was stereotaxically inserted into the target region of the RA. Anodal DC current (500  $\mu$ A, 20 s) was then passed through the insect pin. Lesioning of the opposite target nucleus was performed by the same method. Lesion sites were identified in histological sections of brain tissue.

### Spike recording

Recording electrodes for the spike recording were implanted in 9 males and 6 females. The bird was anesthetized, placed in a stereotaxic instrument, and the window of the skull was opened in the same method as for implanting electrode. To guide the recording electrode to the DM, a vertical hole from the top of the brain to just above the DM was made with a syringe needle. The recording electrode was then inserted vertically into the target nucleus of the DM, and a reference electrode (No.00, Shiga; not insulated) was placed on the skull. The two electrodes were cemented to the skull with dental resin and acrylic glue. The bird was kept warm and recovered in 3–4 hr.

The recording electrode consisted of 4–6 enamel-coated copper wires (diameter: 80  $\mu$ m), joined in parallel straight bundles, and cemented with polyethylene glycol and acrylic glue. One terminal of each wire and each terminal of the IC socket with 8-pins were joined by soldering. The wires and the IC socket were cemented with dental resin and acrylic glue. The edge of the recording electrode was cut with sharp scissors to leave 5 mm of the wire region exposed.

Spike discharges were extracellularly recorded under freely moving conditions in the sound-attenuating box. Two copper wires with high S/N ratio were used in differential recording. To amplify the neural signals, small operational amplifier (TLC082CP, Texas instruments) was set on the bird's head. The operational amplifier was connected to a differential amplifier (AVB-9, Nihon Kohden) with rubber-coated copper wires. Neural signals were monitored on an oscilloscope, low-cut-filtered in 500 Hz, and recorded on a digital audio tape recorder. Distance calls and short calls were induced by play back from a loud speaker. Previously recorded female distance

calls were used as play back call. Induced calls and playback calls were recorded on the digital audio tape recorder through a microphone. More than 10 distance calls and short calls were recorded in each bird. After spike recording was complete, electrolytic lesions were made (DC 20  $\mu$ A, 10 s) at the recording site in the DM. To check the recording site in the DM, histological sections of the brain tissue were made, and observed under a microscope.

Impulse analysis was performed on a digital oscillograph (OMNIACE AT3600, NEC-Sanei) or on a personal computer. Recorded impulses were sliced at certain spike-observed levels (ATAC 350, Nihon Kohden); slice level is more than 3 times of noise levels. The number of sliced impulses was counted around each call vocalization. Spontaneous discharge was defined in a period of randomly selected 50 ms except the 1 sec of call playback.

## RESULTS

### Acoustic structures of induced calls in RA-lesioned male

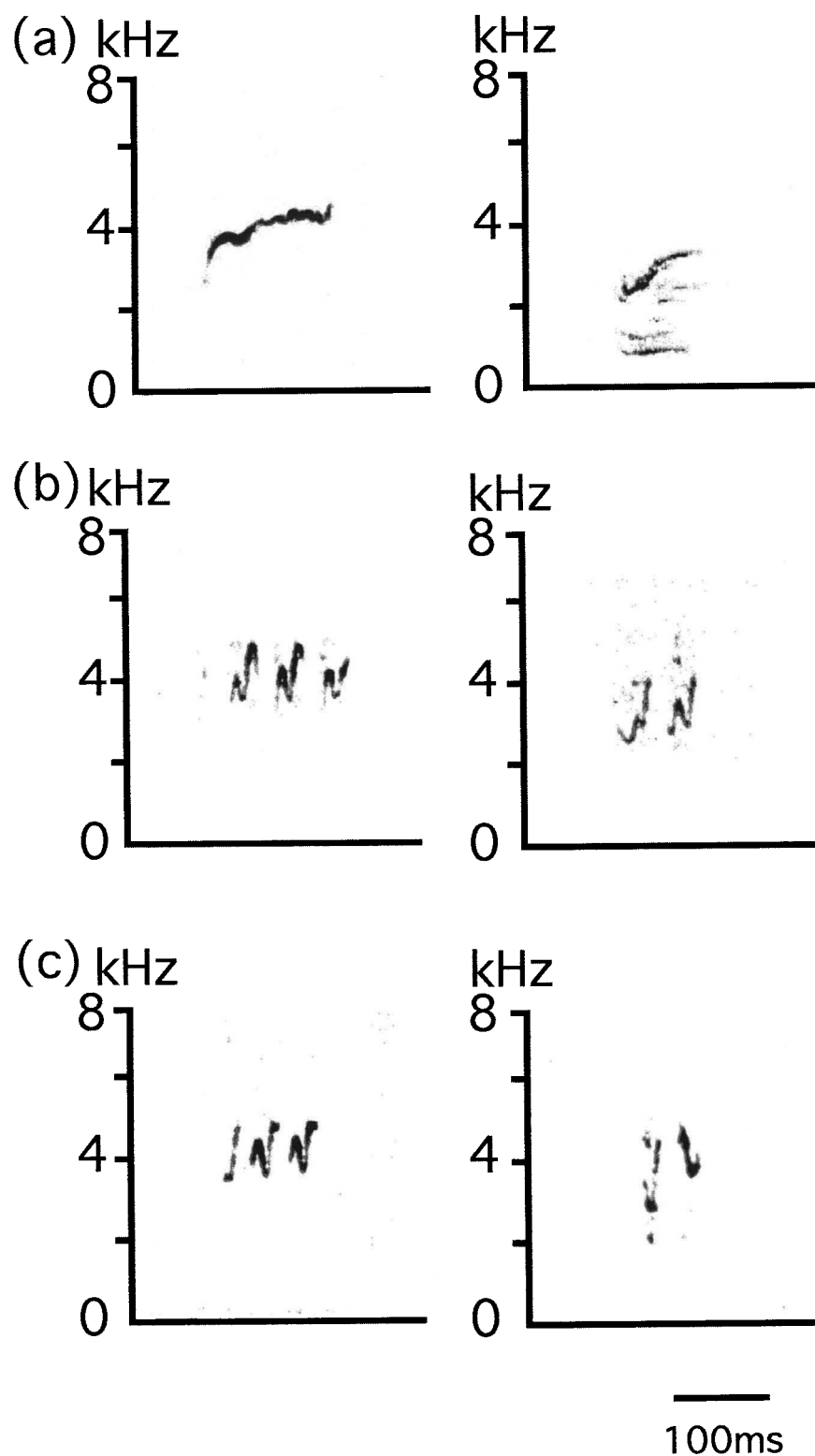
As shown in our previous report (Fukushima and Aoki, 2000), electrical stimulation to the DM of males under specific conditions (80  $\mu$ A, 70–83 Hz, 240 ms) induced a call (Fig. 2 (a), right) acoustically similar to the male distance call (Fig. 2 (a), left). As most of males vocalizes distance calls with 1 note (Fukushima and Aoki, 2000), all calls induced by electrical stimulation (CIES) were consisted of 1 note with gradual frequency modulation in 10 males.

RA-lesioned males vocalized distance calls (Fig. 2 (b), left) acoustically similar to female distance calls (Fig. 2 (c), left); both calls were consisted of multiple notes. In this study, electrical stimulation to the DM of RA-lesioned male under the condition (60–80  $\mu$ A, 70–83 Hz, 240 ms) induced a call with 2 notes and rapid frequency modulation (right of Fig. 2 (b),  $n=7$ ). The number of the notes in the CIES of RA-lesioned males was significantly larger than in the CIES of intact males ( $2.0 \pm 0.0$  vs.  $1.0 \pm 0.0$ ,  $t$ -test and test for equal variance,  $p < 0.01$ ). The pattern of time-frequency characteristics in the acoustic structures of the CIES (Fig. 2 (b), right) was similar to that of the distance calls of RA-lesioned males (Fig. 2 (b), left), but different from that of the distance calls before RA lesions (not shown, but with the similar characteristics to those shown in the left sonogram of Fig. 2 (a)). In RA-lesioned males (Fig. 2 (b), right), the pattern of time-frequency characteristics (number of notes, frequency modulation) in the acoustic structure of the CIES was similar to that of females (Fig. 2 (c), right).

In males, RA-lesioned males, and females, threshold current was 20–60  $\mu$ A. Latency of males, RA-lesioned

**Table 1.** Characteristics of acoustic structures of CIES and distance call (DC)

	n	duration (ms)	n of notes	Highest f (Hz)	Lowest f (Hz)
CIES	male	10 67.8 $\pm$ 7.8	1.00 $\pm$ 0.00	3653 $\pm$ 273	1333 $\pm$ 262
	female	7 78.0 $\pm$ 4.9	2.00 $\pm$ 0.00	4272 $\pm$ 450	1288 $\pm$ 275
	male (RA lesions)	7 80.6 $\pm$ 7.3	2.00 $\pm$ 0.00	3064 $\pm$ 249	1762 $\pm$ 221
DC	male	15 130.7 $\pm$ 5.8	1.06 $\pm$ 0.06	4859 $\pm$ 60	3188 $\pm$ 82
	female	7 160.5 $\pm$ 10.7	3.28 $\pm$ 0.45	4793 $\pm$ 138	3137 $\pm$ 110
	male (RA lesions)	5 116.8 $\pm$ 8.7	2.83 $\pm$ 0.16	4864 $\pm$ 319	3096 $\pm$ 166



**Fig. 2.** Sonograms of distance call (left) and call induced by electrical stimulation (right) to the DM in sexually mature male (a), male after RA lesions (b), and sexually mature female (c).

males, and females was  $206.6 \pm 23.1$  ms,  $217.6 \pm 23.4$  ms, and  $233.4 \pm 18.0$  ms, respectively. Significant difference in the latency of the CIES among the three experimental groups was not observed.

As noted in males, RA-lesioned males, and females, acoustic structures of CIES were partly similar to those of the distance call, and partly different. Table 1 shows characteristics of acoustic structures of CIES (80  $\mu$ A, 71 Hz, 240 ms) and of distance calls under natural conditions. In all groups, highest and lowest frequencies of CIES were lower than those of distance calls. Duration of CIES was shorter than that of distance calls in all groups. In RA-lesioned males and females, the CIES contained fewer notes than the distance call.

#### Acoustic structures of induced calls under different conditions

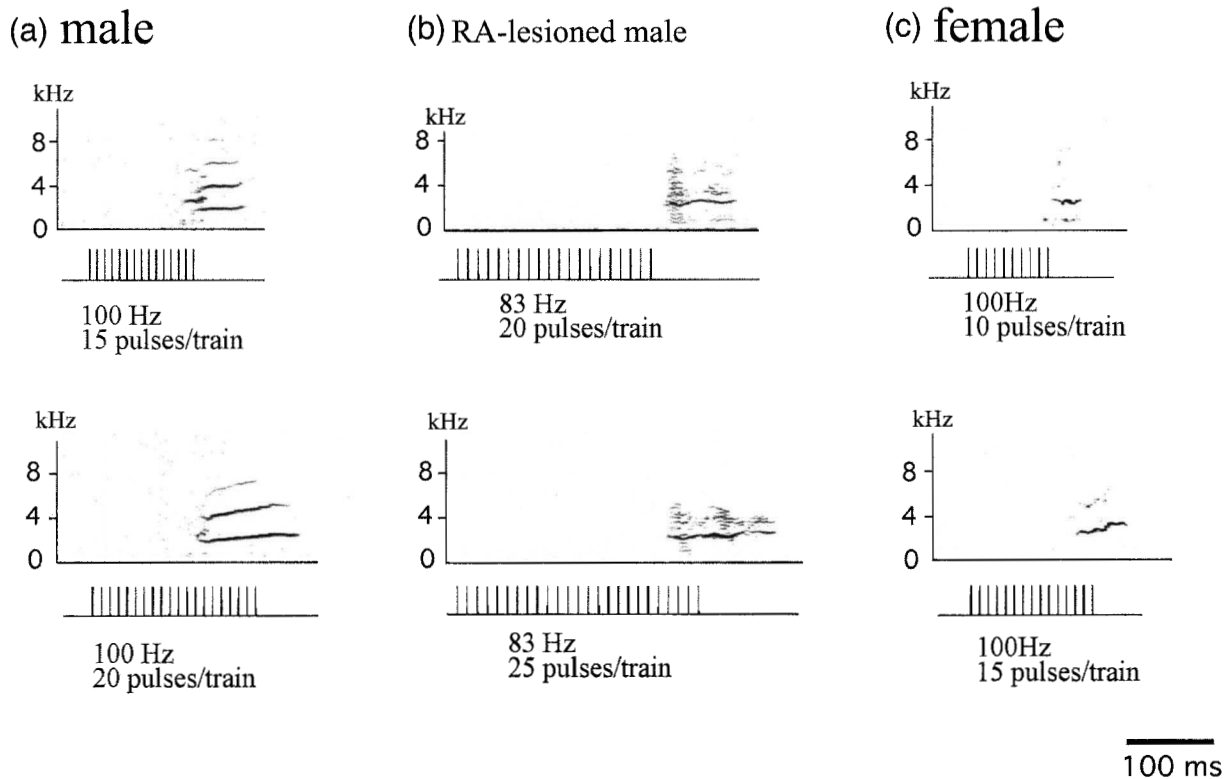
Electrical stimulation under different conditions (pulse frequency, number of pulses/train, and train duration) was performed on the DM of both sexes in order to clarify the sexual difference of the endogenous ability of the neural circuit in the DM. One parameter was fixed at a time, because fixing two of three parameters automatically fixes the remaining of one. Under each condition, the pulse duration and pulse current was fixed (500  $\mu$ s and 80  $\mu$ A, respec-

tively). In each individual, every time of electrical stimulation under the same conditions, CIES show almost similar acoustic structures.

##### (1) Stimulation at constant frequency

Electrical stimulation was performed on the DM at constant frequency (within 70–100 Hz) for several train durations (mainly 100–400 ms) and several numbers of pulses/train (mainly 10–30). In males ( $n=10$ ), RA-lesioned males ( $n=7$ ) and females ( $n=7$ ), electrical stimulation for a relatively long train duration (relatively large number of pulses/train) produced a call with relatively long duration (bottom of Fig. 3 (a)–(c)), and electrical stimulation for a relatively short duration (relatively small number of pulses/train) produced a call of relatively short duration (top of Fig. 3 (a)–(c)). Electrical stimulation for very short duration (too small number of pulses/train; shorter than the constant value (5–15) pulses/train for each bird) did not produce a call. Electrical stimulation with excessively long train durations (more than the constant value (25–40) pulses/train for each bird) produced calls more than twice.

When the train duration was increased or decreased, the pattern of time-frequency in the acoustic structure of CIES was sometimes changed. However, a constant relation between the length of train duration and the pattern of time-frequency of CIES was not observed.

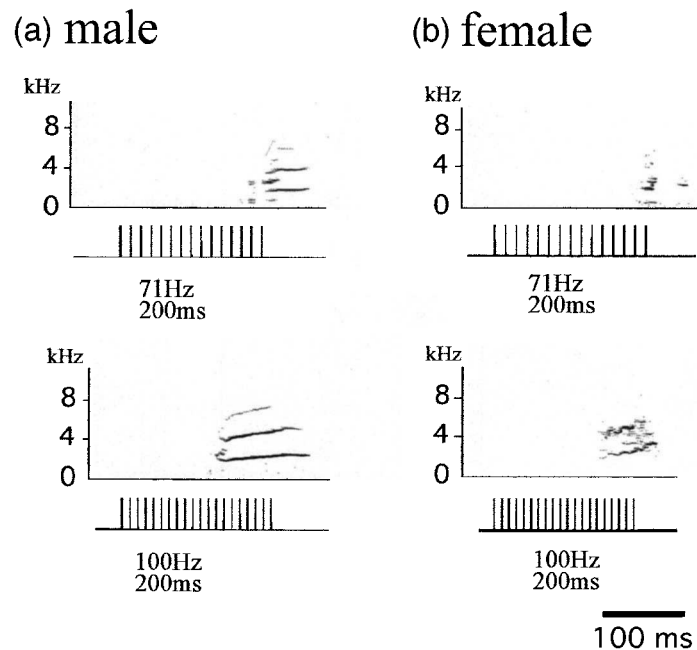


**Fig. 3.** Sonograms of calls induced by electrical stimulation (CIES) at constant frequency in male (a), male after RA lesions (b), and female (c). In (a), (b), and (c), top sonogram shows CIES for relatively small number of pulses/train (relatively short train duration), and bottom sonogram shows CIES for relatively large number of pulses/trains (relatively long train duration). Waveform beneath each sonogram indicates the timing and the number of the pulses of electrical stimulation. Pulses were 80  $\mu$ A.

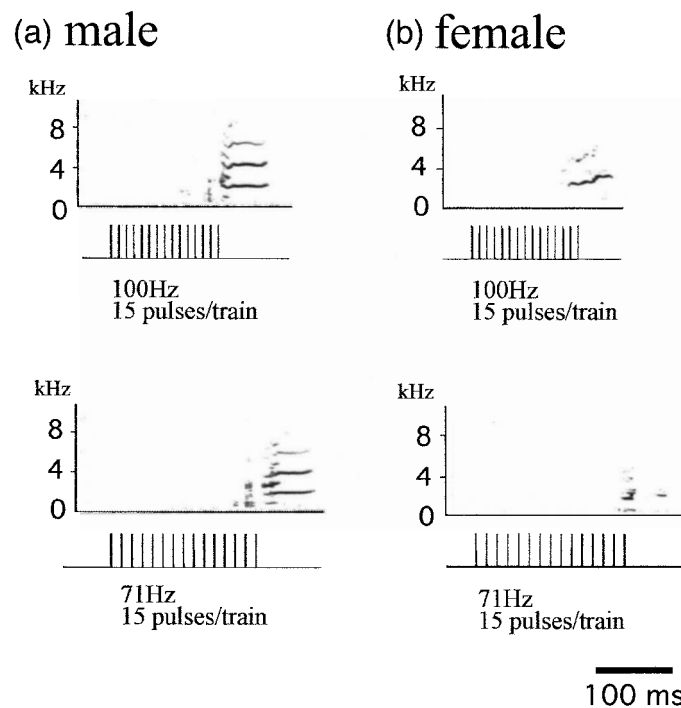
## (2) Stimulation at constant train duration

Electrical stimulation was performed on the DM for constant train duration (within 150–300 ms) and several num-

bers of pulses/train (mainly 10–30) at several frequencies (mainly 70–100 Hz). In males (Fig. 4 (a)), RA-lesioned males (not shown) and females (Fig. 4 (b)), relatively high fre-



**Fig. 4.** Sonograms of CIES for constant train duration in male (a), and female (b). In (a) and (b), top sonogram shows CIES at relatively low frequency (relatively small number of pulses/train), and bottom sonogram shows CIES at relatively high frequency (relatively large number of pulses/train). Waveform beneath each sonogram indicates the timing and the number of the pulses of electrical stimulation as in Fig.3. Sonograms (a) and (b) were recorded from the same individuals as shown in Fig.3 (a) and (c) respectively.



**Fig. 5.** Sonograms of CIES for constant number of pulses/train in male (a), and female (b). In (a) and (b), top sonogram shows CIES at relatively high frequency (relatively short train duration), and bottom sonogram shows CIES at relatively low frequency (relatively long train duration). Waveform beneath each sonogram indicates the timing and the number of the pulses of electrical stimulation as in Fig.3. Sonograms (a) and (b) were recorded from the same individuals as shown in Fig.3 (a) and (c) respectively. In the top and bottom of sonogram (a), the parameters of stimulation was the same as in the top sonogram of Fig.3 (a) and the top sonogram of Fig. 4 (a), respectively.

quency stimulation (relatively large number of pulses/train) produced a call with a relatively long duration (bottom of Fig. 4 (a)–(b)), and electrical stimulation at a relatively low frequency (relatively small number of pulses/train) produced a call with relatively short duration (top of Fig. 4 (a)–(b)). Electrical stimulation at less than 70 Hz did not produce a call.

In males, electrical stimulation at any frequency produced a call with 1 note (Fig. 4 (a)). In RA-lesioned males and females, electrical stimulation at low frequency (71–85 Hz) produced a call with several notes (top of Fig. 4 (b)), and electrical stimulation at high frequency (<85 Hz) produced a call with 1 note (bottom of Fig. 4 (b)).

### (3) Stimulation at constant numbers of the pulses/train

Electrical stimulation was performed on the DM with constant number of pulses/train (within 15–30) for several train durations (mainly 150–400 ms) at several frequencies (mainly 70–100 Hz). As shown in Fig. 5, a constant relation between the frequency of electrical stimulation and duration of CIES was not observed.

In males, electrical stimulation at any frequency produced a call with 1 note, but it did not produce a call with multiple notes (Fig. 5 (a)). The number of notes was not dependent on the frequency of electrical stimulation in males. In females (Fig. 5 (b)) and RA-lesioned males (not shown), electrical stimulation at low frequency (71–85 Hz) produced a call with multiple notes (bottom of Fig. 5 (b)), and electrical stimulation at high frequency (85–100 Hz) produced a call with 1 note (top of Fig. 5 (b)). Electrical stimulation at the border frequency (around 85 Hz) induced calls with 1 note and 2 amplitude peaks. In females and RA-lesioned males, electrical stimulation with very narrow band width current (60–80  $\mu$ A in each bird) produced a call with multiple notes. Electrical stimulation at 70–85 Hz with too much current (>80  $\mu$ A) and too little current (<60  $\mu$ A)) pro-

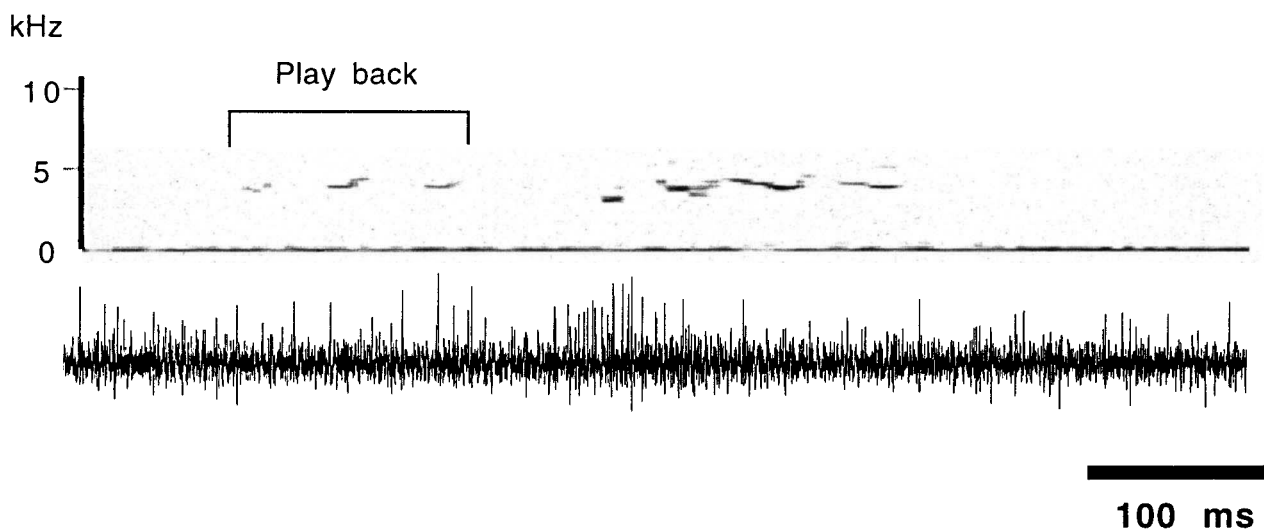
duced a call with 1 note and long duration (40–80 ms) and short duration (10–20 ms), respectively. In females and RA-lesioned males, the acoustic difference of CIES in different frequencies was larger than that in intact males.

### Relation between the distance calls and spike discharges

Nerve impulses were recorded from neurons in the DM of 9 males and 6 females. As shown in Fig. 6, the level of spike discharge in the DM before and during distance call vocalization was higher than the spontaneous discharge level in males and females. In all cases (9 males and 6 females), the temporal relation between the spike discharges in the DM by playback call stimulation and the responding distance call was as follows. Spike discharges in the DM occurred after about 230–400 ms of delay following playback call stimulation. After 30–50 ms delay following spike discharges in the DM, the bird emitted a distance call. When the bird did not emit any kind of call, the number of spike discharges from DM neurons did not increase.

After playback call stimulation, birds of both sexes sometimes emitted short calls. The male short call consisted of 1 note with simple upward frequency modulation, while the female short call consisted of 1 note—sometimes 2—with simple upward frequency modulation. In 4 males and 4 females, the total number of spike discharges with the short call increased about 1.5 times more than the total number of spontaneous discharges (not shown). In 1 male, the change of firing rate of spike discharges in the DM did not elicit a short call. The remaining 4 males and 2 females did not emit short calls during recording session.

To quantify temporal patterns of spike firing, the number of spikes per 50 ms associated with vocalization of 11 distance calls was counted and averaged in each bird. Fig. 7 (a) shows an example of spike firing pattern of DM neurons

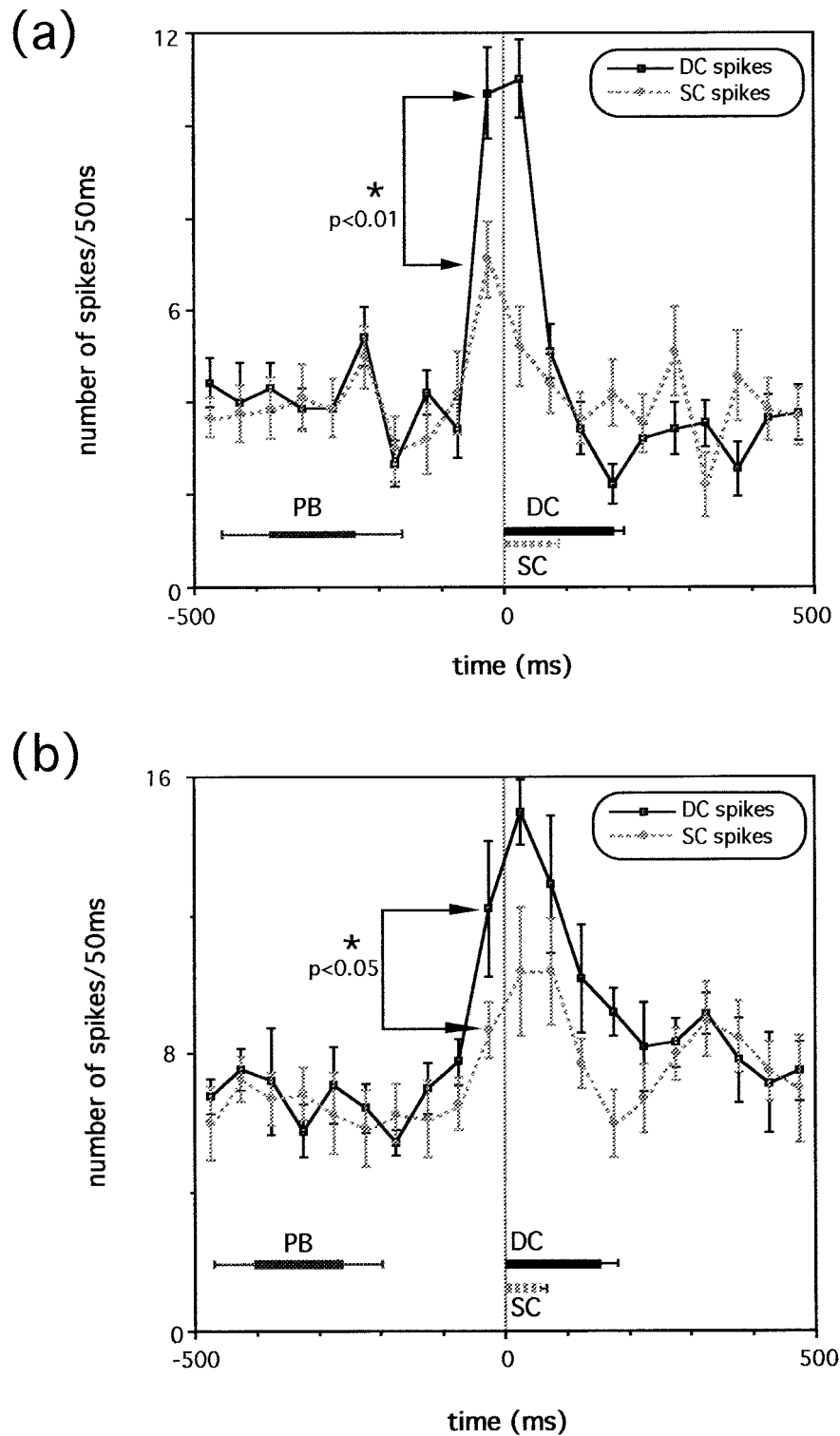


**Fig. 6.** An example of spike discharges related to vocalization of distance call (a) and short call (b), recorded from neurons in the DM of a male. Sonogram above each trace indicates playback call (left) and emitted distance call (right).



in response to play back stimulation in a male. In the male shown in Fig. 7 (a), mean number of spikes increased 258% immediately before the distance call was emitted. Mean

number of spikes just before emitting the distance call ( $-50 - 0$  ms, nDC) was significantly larger than that of spontaneously discharged spikes (nSP, t-test and test for equal vari-



**Fig. 7.** Impulse frequency over time for neurons in the DM related to vocalization of distance call and short call in males (a) and females (b). Onset of distance call (or short call) is defined as 0 ms. Left horizontal line indicates the timing of distance call playback (PB). Right horizontal lines indicate mean duration of distance call (DC) and short call (SC). Y-axis indicates mean frequency of identified impulses per 50 ms. Error bar indicates standard error.

ance,  $p < 0.01$ ). The mean number of discharged spikes just before an emitted short call was 166% greater than in spontaneous discharge. The mean number of spikes just before the short call (nSC) was significantly larger than that of spontaneously discharged spikes ( $p < 0.01$ ). As shown in Fig. 7 (a), mean number of spikes just before an emitted distance call was 176% larger than that just before an emitted short call ( $p < 0.01$ ). In 4 males, the mean number of spikes from the discharges just before an emitted distance call / mean number of spontaneously discharged spikes (nDC / nSP) was  $198 \pm 23\%$  (mean  $\pm$  SE), mean number of spikes from the discharges just before an emitted short call / mean number of spontaneously discharged spikes (nSC / nSP) was  $134 \pm 18\%$ , and mean number of spikes from the discharges just before an emitted distance call / mean number of spikes from the discharges just before an emitted short call (nDC / nSC) was  $152 \pm 15\%$ .

Fig. 7 (b) shows an example of the response of spike discharge of DM neurons in a female. As shown in Fig. 7 (b), nDC was significantly larger than nSP (189% increased,  $p < 0.05$ ), nSC was significantly larger than nSP (144% increased,  $p < 0.05$ ), and nDC was significantly larger than nSC (175% increased,  $p < 0.05$ ). In 4 females, nDC / nSP is  $212 \pm 15\%$ , nSC / nSP is  $129 \pm 10\%$ , and nDC / nSC is  $179 \pm 2\%$ . The response curves of the numbers of spike discharge neurons in the DM of both males and females (shown in Fig. 7 (a) and (b)) show almost the same pattern.

## DISCUSSION

In our study, electrical stimulation of several different parameters was delivered to the DM in order to clarify the neural function of the neural system in the DM. First, calls induced by electrical stimulation (CIES) of different numbers of pulses/train were analyzed. In sexually mature males and females, electrical stimulation of relatively large number of pulses/train in constant duration (or in constant frequency) produced a call with relatively long duration, and electrical stimulation with relatively small number of pulses/train in constant duration (or in constant frequency) produced a call with short duration. To identify that these processes were observed in the neural circuit of the DM corresponding with distance call vocalization, spike discharges from DM neurons were recorded in sexually mature males and females under freely moving conditions. In males and females, the bird vocalized distance calls following the large increment of impulses or short calls following the small increment. The mean number of spike discharges before emission of a distance call was significantly larger than that before emission of a short call. These results suggest that the neural system in the DM of both sexes controls duration of two calls (short calls, distance calls) depending on the number of impulses as motor pattern generator.

According to the hypothesis by Simpson and Vicario (1990), the neural system in the DM shows sexually similar function and the neural input from the RA to the DM

depresses call vocalization during singing. Our previous results (Fukushima and Aoki, 2000) expanded the hypothesis of Simpson and Vicario in production of male distance calls, that the neural system in the DM shows sexually different function and neural input from the RA to the DM controls male-specific elements of distance calls (number of notes, frequency modulation, etc).

To identify the neural function of the DM controlling acoustic structures of distance calls (frequency modulation, number of notes, etc) in physiological methods, CIES at different frequencies were analyzed in sexually mature males, females, and RA-lesioned males. As shown in previous reports using some avian species (Potash, 1970; Seller, 1981), electrical stimulation to the DM at different frequencies induced calls with different acoustic structures with patterns of time-frequency. We found that in sexually mature females electrical stimulation to the DM at 100 Hz produced a call with 1 note, and electrical stimulation at 70 Hz produced a call with 2 notes. These results suggest that the neural system in the DM of females controls not only duration but also frequency modulation in call vocalization.

Electrical stimulation to the DM of sexually mature males at any frequency induced a call with 1 note. In RA-lesioned males, electrical stimulation to the DM induced a call with 1 or 2 notes as in that of females. In addition to these results, neural connection from the RA to the DM is unidirectional in sexually mature males (Wild, 1997; Striedter and Vu, 1998; Fukushima and Aoki, 2000), and latency to induce the call in RA-lesioned males was almost same as that for intact males. Our results of electrical stimulation in males, females, and RA-lesioned males could not be explained by the hypothesis that the function of the DM is sexually similar (Simpson and Vicario, 1990). Our results suggest that the neural system of the DM in Bengalese finches shows sexually different functions for emitting distance calls. Because anatomical and functional difference of the neural circuits in the DM between both sexes is due to the neural input from the RA to the DM, our results confirmed that the neural system in the DM of both sexes can act as motor pattern generator corresponding with sexually different acoustic structures of distance calls.

Our results of electrical stimulation suggest that the neural system of the DM have sexually different function corresponding with distance call vocalization, and the process of distance call production is thought to show sexually different manner. However, sexual difference of temporal pattern of spike discharges was not observed. Our results of spike recording could not directly support our hypothesis. To identify the sexual difference of the physiological process of the neural circuit in the DM for distance call vocalization, further detailed study is needed. For example, single-unit spike recording from the DM neurons *in vivo*, or intracellular recording from the neurons in the DM *in vitro* may show these differences.

Acoustic differences of distance calls exist between the two species, zebra and Bengalese finches, although both

species have anatomically same call control system. Acoustic structures of distance calls in Bengalese finches are more complex than those in zebra finches, because frequency modulation is acoustically observed in distance calls of Bengalese finches, but not observed in those of zebra finches. Sexual difference of acoustic structures of distance calls were large in Bengalese finches, but small in zebra finches; The difference is observed in the number of notes and temporal pattern of time-frequency characteristics in Bengalese finches, and in only replacement of noise element in zebra finches. Moreover, there is a song element (syllable) acoustically similar to their own distance calls in male zebra finches, but not observed in male Bengalese finches. As behavioral difference between the two species is observed, there is a possibility that the neural circuit in the DM is functionally different between the two species.

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