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The Role of the Dorsomedial Nucleus (DM) of Intercollicular Complex with Regard to Sexual Difference of Distance Calls in Bengalese Finches

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ABSTRACT—The acoustic structures of distance calls in sexually mature Bengalese finches (*Lonchura striata* var. *domestica*) show distinct sexual differences. We conducted a neuroethological study to identify the neural mechanism in the brain by which sexually mature male and female birds produce these acoustically different distance calls. Bilateral lesions of the dorsomedial (DM) nucleus of the intercollicular complex, known as the midbrain vocal center, eliminated distance calls in sexually mature males and females, but electrical stimulation of the DM in males and females induced calls that showed sexual differences and were acoustically similar to the distance calls of males and females. These results confirm that the DM in both sexes is one of the nuclei of the vocal control system that controls calling behavior. Neural tracer was injected into the DM in order to identify sexual differences in the neural input and output connections in the DM. Anatomical tracing of DM revealed the existence of labeled somata in the ipsilateral robust nucleus of the archistriatum (RA) in males. After bilateral RA lesions, however, males produced distance calls that were acoustically similar to female distance calls. These results suggest that the DM is one of the nuclei which generate the distance call patterns of both sexes, and that the neural information from the RA to the DM change the distance call pattern of male.

INTRODUCTION

Passerine birds emit two large types of vocalizations, songs and calls. (Konishi, 1994). In passerines, sexually mature males sing songs consisting of long and complicated sounds, but females do not. Both males and females, however, produce calls of brief and simple sounds. Distance call is produced when a passerine bird is visually separated from its conspecifics (Zann, 1985). In sexually mature male and female Bengalese finches (*Lonchura striata*, var *domestica*), the acoustic structures of distance calls show sexual differences (Okanoya and Kimura, 1993), but immature males and females have acoustically similar distance calls (Yoneda and Okanoya, 1991).

The dorsomedial (DM) nucleus of the intercollicular complex is one of the nuclei of the vocal control system and is known as the midbrain vocal center (Seller, 1981). In many avian species (Java sparrow, red-winged blackbird, Japanese quail, zebra finch, and others), electrical stimulation of the DM induces calls acoustically similar to distance calls (Brown, 1965a; Potash, 1970; Seller, 1980; Vicario and Simpson, 1995), and bilateral lesioning of the DM eliminates the calls (Brown, 1965b; Seller, 1981). The possible role of DM in call

production has been well investigated in non-oscine birds, but not sufficiently in oscine birds.

Sexually mature male Bengalese finches have a song control system, but females do not (Nottebohm and Arnold, 1976). In neural substrates for song, the nucleus nervi hypoglossi, the tracheosyringealis nucleus (nXIIts), receives neural output from the DM, and the DM receives neural input from the robust nucleus of the archistriatum (RA) of the forebrain. The neurons in nXIIts directly innervate the avian vocal organ, syrinx. The RA lesions disrupt song in mature male canaries (Nottebohm *et al.* 1976). The vocal control system incorporates neural mechanism which produces the male distance call. The vocal control system incorporates a neural mechanism which produces the male distance call. After bilateral RA lesions, however, sexually mature male zebra finches produce distance calls that are acoustically similar to those of females (Simpson and Vicario, 1990).

The purpose of this study is to clarify the physiological function of the DM in the vocal control system and in the production of sexually different acoustic structures of distance calls. First, we investigated the physiological function of the DM with electrolytic lesions and electrical stimulation to the DM of both sexes in order to confirm that the DM is indeed one of the call nuclei. Second, we performed bilateral or unilateral lesions of the RA which have a neural pathway to the DM in males.

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MATERIALS AND METHODS

Sexually mature Bengalese finches (25 male, 19 female) were used in this study. All birds were purchased from a commercial source and caged in groups of 3–15 individuals at 22–28 degrees.

Bird calls recording and analysis

Distance calls were induced by the playback method. Recording was performed in a simple sound-attenuated box using an acrylic water bath insulated with sponge rubber lining. Distance calls were recorded on a digital audio tape recorder (TCD-D100, Sony) through a microphone (CM-60, AIWA). Acoustic analysis was performed with a sound spectrograph (DSP Sona-Graph Model 5500, Kay Electronics Corp.). A distance call was defined as a vocalization surrounded by at least 50 ms of silence whose duration was more than 100 ms. A note in a call was defined as the continuous sound between intervals. By these definitions, total duration, number of notes, and the highest and lowest frequencies of each distance call were measured on a sound spectrogram. In this experiment, the frequency modulation of the distance call was measured as follows. On the sound spectrogram, the points were joined by a line when the trace was continuous. To treat upward and downward frequency modulation as the same, the absolute value of the slope of the frequency (AVSF) was measured in each line. To reflect each length of duration of the value of AVSF, the frequency modulation of the distance call was measured as follows.

$$\frac{\sum \{(\text{each value of AVSF}) \times (\text{duration of each value of AVSF})\}}{(\text{total duration of the call}) - \{\sum (\text{duration of intervals between notes})\}}$$

Lesions

Bilateral DM lesions were performed in 3 males and 3 females. Bilateral and unilateral RA lesions were performed in 4 males and 2 females, respectively. Distance calls in each bird were recorded 1–3 days before lesioning. After the bird was deeply anesthetized with a mixture of ketamine and xylazine, it was placed in the stereotaxic instrument. The skin was opened, and a window in the top of the skull was opened over the appropriate brain area. A stereotaxic atlas (Konishi, unpubish data) was used to identify a target nucleus. An insect pin electrode (Shiga, No.00; insulated except at the tip, 120–150 μm) was vertically inserted into the region of the target nucleus. Anodal DC current (500 μA , 20s) was passed through the electrode. Lesioning of the target nucleus in the other side of the brain was performed by the same method when needed. After the skin of the head was sealed as before, the bird was kept warm and allowed to recover for 4–5 hr. Distance calls were recorded 3 days, 7 days, and 28 ± 3 days after the lesions.

After final distance call recording, the bird was deeply anesthetized by injection with sodium pentobarbital. The bird was transcardially perfused with 30 ml of heparinized 0.01 M phosphate buffered saline and with fixative. The brain was embedded in 15% gelatin and cytoprotected in 15% sucrose solution in 0.1 M phosphate buffer. The brain was then frozen and sectioned frontally at 50 μm . Sections were mounted on the slide, dried, and stained with cresylviolet acetate. Each tissue preparation was observed under a microscope to check the lesion sites.

Electrical stimulation

Electrical stimulation was performed on 6 males and 4 females. After preparation by the same method as in the lesion experiment, an insect pin electrode (Shiga, No. 00; insulated except at the tip 10–30 μm) was vertically inserted into the region of the DM, cemented to the skull with dental resin, and built up with acrylic glue. After the bird had recovered for 3–4 hr, electrical stimulations were performed under free-moving conditions. Several anodal current pulses (500 μs duration, $<150 \mu\text{A}$) were administered at several frequencies. The induced

vocalization and timing of electrical stimulations were recorded on a personal computer (PC-9801VX, NEC) through an oscilloscope (VC-9, Nihon-Kohden). Acoustic analysis was performed with a sound spectrograph. To locate the electrode tip site, anodal current (DC 10 μA , 10 s) was passed through the electrode after electrical stimulation. Brain sections of 50 μm were made in the same manner as in the lesion experiment. The electrode tip site was then stained with 1% potassium ferrocyanide in 1% HCl. Sections of the tissue were counterstained with neutral red and observed under a microscope.

Neural tracers

Neurobiotin (Sigma) or biocytin (Sigma) were used as neural tracers. Neurobiotin was injected into the DM in 4 males and 5 females and into the RA in 2 males. Biocytin was injected into the DM in 2 males and 1 female and into the RA in 4 males. After preparation as in the lesion experiment, 5% neurobiotin or biocytin in 0.5 M KCl was injected iontophoretically (biphasic current pulses from 5 to $-2 \mu\text{A}$ [neurobiotin] or from 20 to $-5 \mu\text{A}$ [biocytin], 2 Hz, 10 min) through a glass micropipette (tip diameter 20–30 μm). After survival periods of 4–6 hr (neurobiotin) or 9–11 hr (biocytin), 50 μm sections of the brain were made as in the lesion experiment. Neurobiotin or biocytin labeling was visualized with ABC standard kits (Vector) and with a nickel- and cobalt-intensified DAB reaction. Sections were counterstained with neutral red and observed under a microscope.

RESULTS

Acoustic structures of distance calls between sexually mature males and females

Typical distance calls in sexually mature males and females are shown in Fig. 1. Acoustic structures of distance calls in both sexes did not show harmonic structures. Quantitative analysis of distance calls was performed in 6 males and 6 females (Fig. 7). Mean number of the notes of male and female calls was 1.2 and 3.5, respectively, but the mean number of male notes was significantly smaller than that of females (t -test, $p < 0.01$). Mean frequency modulation of male and female distance calls was 15.5 Hz/ms and 41.1 Hz/ms, respectively, and the mean frequency modulation of the male distance call was significantly greater than that of the female ($p < 0.01$). There were no significant differences in total duration nor in the highest and lowest frequencies between both sexes. The distance call of a typical male consisted of one note with gradual frequency modulation, and that of the typical female was 3–5 notes with rapid frequency modulation. The acoustic structures of distance calls in sexually mature males and females were different, so it did show sexual difference.

Lesions and electrical stimulation to the DM

After bilateral DM lesions, neither males nor females produced distance calls. Distance calls were eliminated beginning just after recovery from surgery. Electrical stimulation to the site in the DM of both sexes induced calls at 60–250 ms latency. The lowest threshold was 20 μA , and typical threshold was 40 μA . As shown in Fig. 2, electrical stimulation to the DM under these conditions (80 μA , 83 Hz, 240 ms) induced calls of one note in the male and a few notes in the female. Duration was 100–130 ms. The acoustic structure of the call was similar to that of the distance call. Some acoustic ele-

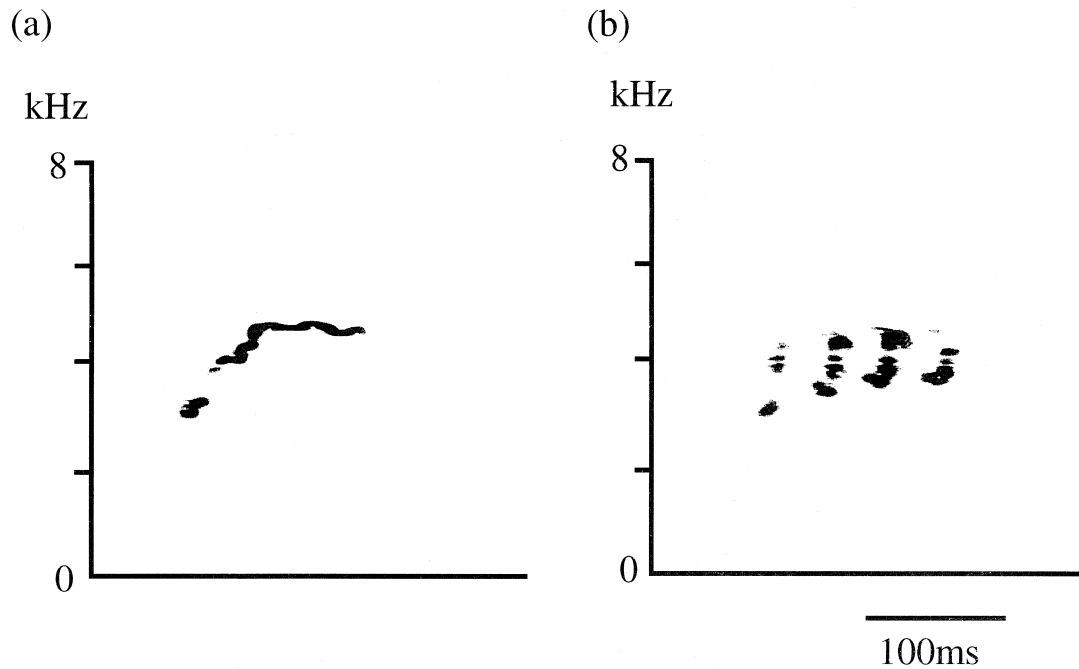


Fig. 1 Sonograms of distance calls in sexually mature male (a) and female (b) Bengalese finches.

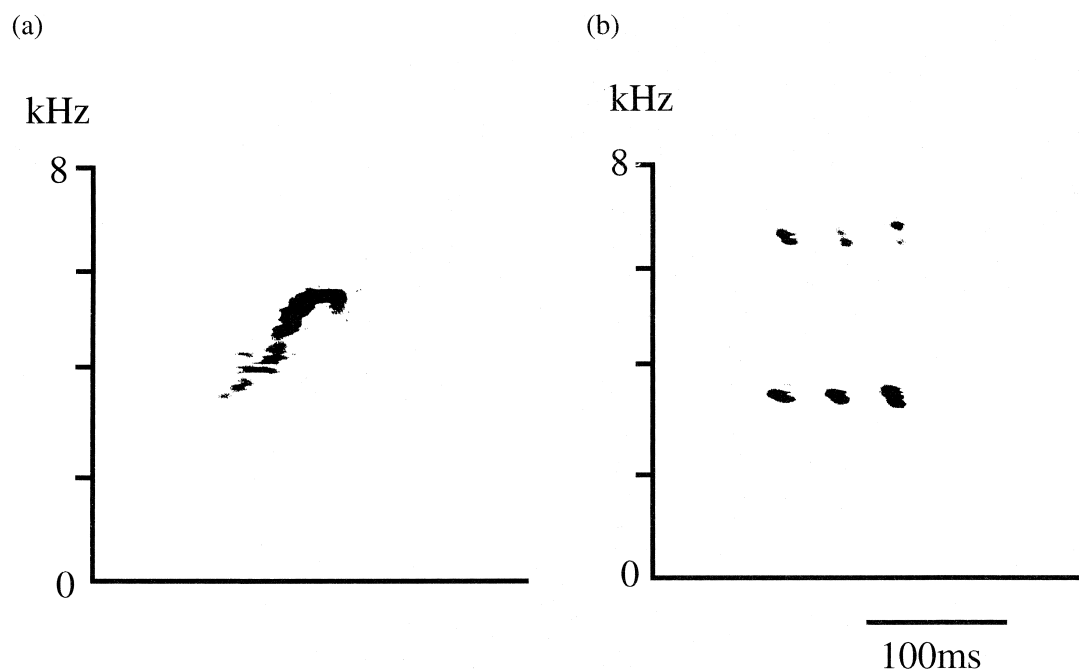


Fig. 2 Sonograms of calls in sexually mature males (a) and females (b) induced by electrical stimulation of DM.

ments (number of notes, temporal pattern of frequency modulation) in the call induced by electrical stimulation to the DM of sexually mature males and females were similar to those of the distance call. Duration of the electrically induced call was shorter than that of the normal distance call.

Neural pathways from/to DM

After neurobiotin was injected into the DM of 4 males, labeled somata were observed in the ipsilateral RA (3/4), the

ipsilateral hypothalamus (3/4), and the contralateral DM (2/4), as shown in Fig. 3(a)–(c). After the injection of biocytin into the DM of 2 males, labeled terminals were observed in the ipsilateral hypothalamus, contralateral DM, bilateral nucleus uvaformis (Uva), and bilateral nXIIIs, as shown in Fig. 3(d). After neurobiotin (n=4) or biocytin (n=2) injection into the RA of males, labeled terminals were observed in the ipsilateral DM, but not in the contralateral DM (Fig. 4).

Neurobiotin injection into the DM of 5 females labeled

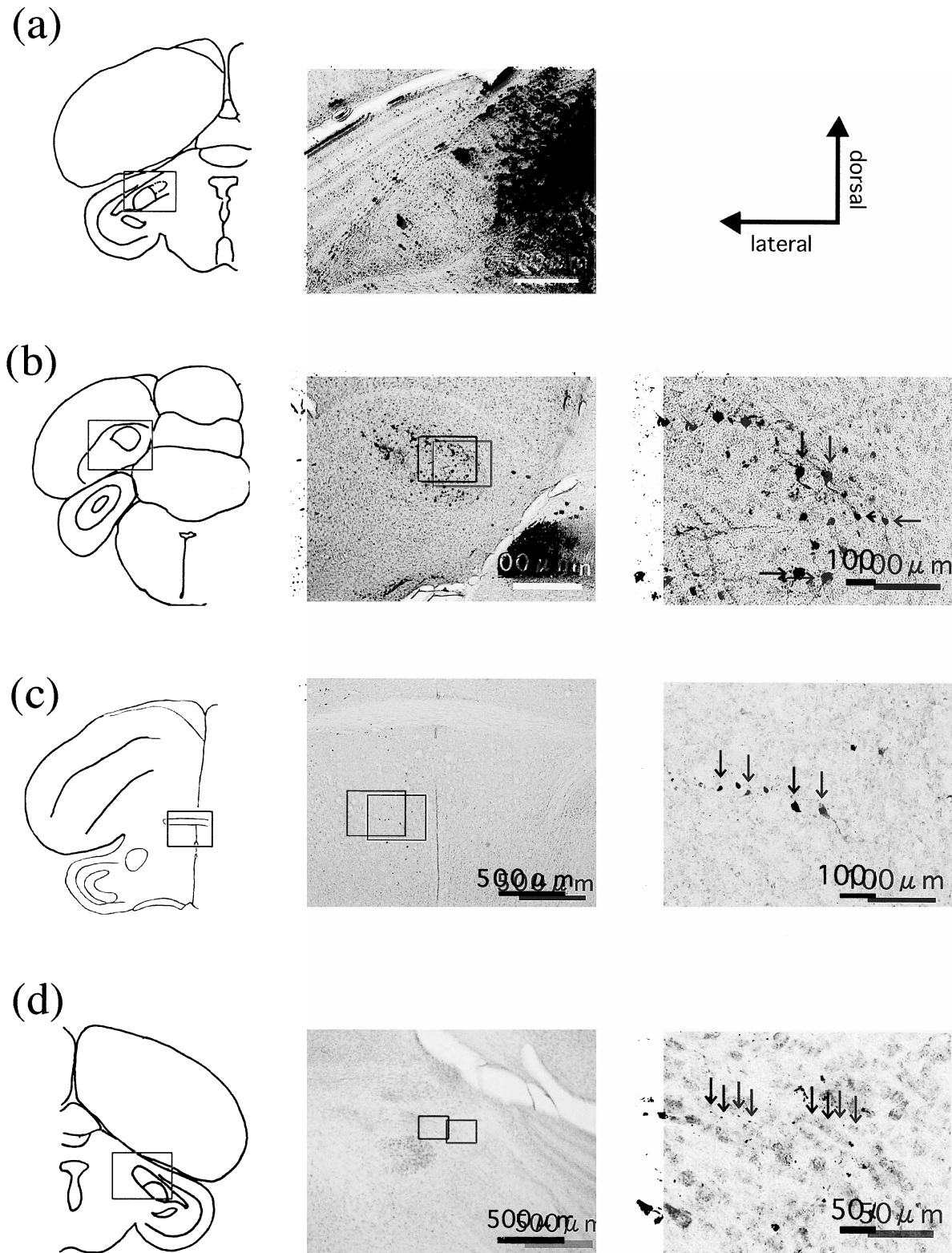


Fig. 3 Results of tracer injections into the DM in sexually mature males. Left schematic drawing (a) shows DM in the frontal section of the brain. Right photomicrograph shows the results of a tracer injection into DM. Left schematic drawings (b) and (c) show the RA and hypothalamus in the frontal section of the brain. Middle photomicrographs (b) and (c) show labeled somata in the ipsilateral RA and in the ipsilateral hypothalamus. Right (higher magnification) images (b) and (c) reveal labeled somata in the ipsilateral RA and ipsilateral hypothalamus. Left schematic drawing (d) shows contralateral DM in the frontal section of the brain. Middle photomicrograph (d) shows contralateral DM. Right higher magnification (d) reveals labeled terminals in contralateral DM. Arrows in right photomicrographs (b) and (c) indicate labeled somata; arrows in (d) indicate labeled terminals.

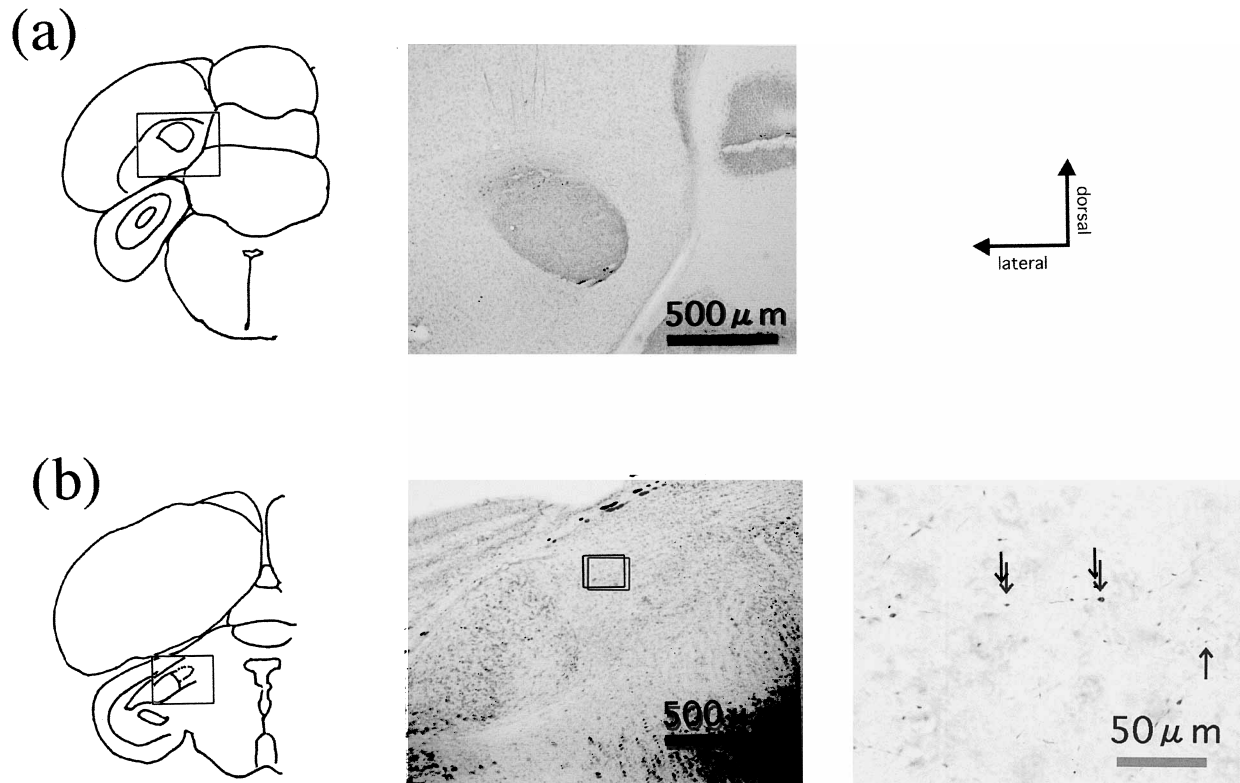


Fig. 4 Results of a tracer injection into RA in a sexually mature male. Left schematic drawing (a) shows RA in frontal section of the brain. Right photomicrograph shows a biocytin injection site in RA. Left schematic drawing (b) shows DM in the frontal section of the brain. Rectangle in middle photomicrograph (b) shows the ipsilateral DM. Right higher magnification (b) reveals labeled terminals in ipsilateral DM. Arrows in the right photomicrograph (b) indicate labeled terminals.

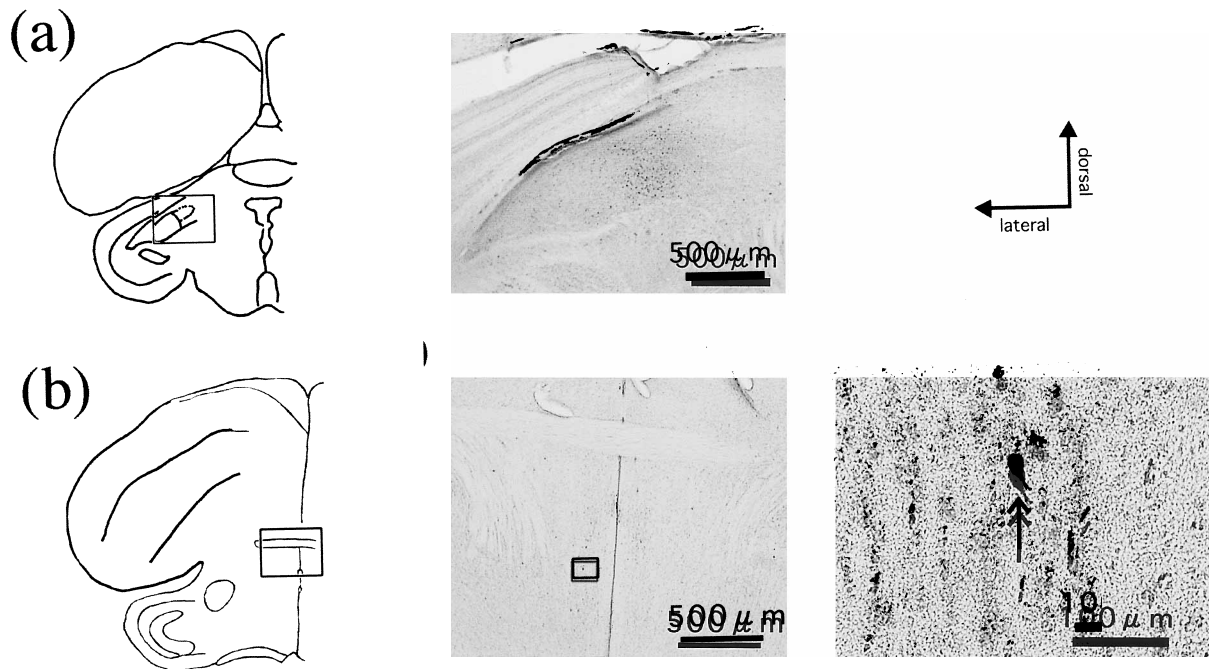


Fig. 5 Results of a tracer injection into DM of a sexually mature female. Left schematic drawing (a) shows DM in the frontal section of the brain. Right photomicrograph shows a neurobiotin injection site in DM. Left schematic drawing (b) shows the hypothalamus in frontal section of the brain. Rectangle in middle photomicrograph (b) shows hypothalamus. Right higher magnification (b) reveals labeled somata in ipsilateral hypothalamus. Arrow in the right photomicrograph (b) indicates labeled somata in hypothalamus.

somata in the ipsilateral hypothalamus (4/5) and contralateral DM (2/5), as shown in Fig. 5. Biocytin injection into the DM of 1 female labeled terminals in the ipsilateral hypothalamus, contralateral DM, bilateral Uva, and bilateral nXIIIs.

Neural input from the RA to the DM was observed in the male but not in the female, but the neural outputs from DM to RA were not observed in either sex. No obvious differences were observed in the region of labeled somata and terminals when neural tracer was injected into the left or right side of the brain.

Distance calls before and after RA lesions

Differences in the acoustic structures of distance calls in males before and after bilateral RA lesions are shown in Fig. 6(a). As shown in Fig. 7, mean number of notes in the distance call after bilateral lesioning was significantly larger than before it (1.3 to 3.3, $p < 0.05$, paired t-test), and the mean frequency modulation of distance calls after bilateral lesioning was significantly higher than before it (15.2 to 41.2 Hz/ms, $p < 0.01$, paired t-test). There were no significant differences in

total duration nor in the highest and lowest frequencies between distance calls before and after RA lesions. After bilateral lesioning was performed in males, mean frequency modulation and number of notes were similar to those of females. After bilateral RA lesions, males also produced distance calls acoustically similar to those of females. The acoustic structure of the male distance call changed just after recovery from surgery. There were no obvious differences among the acoustic structures of distance calls recorded 3 days, 7 days, and 28 days after bilateral RA lesions.

Acoustic structures of distance calls before and after unilateral RA lesioning differed as shown in Fig. 6(b). In distance calls after unilateral lesioning, the mean number of notes was 1.8 and mean frequency modulation was 27.4 Hz/ms, as shown in Fig. 7. These two acoustic elements were the mid-values between those of normal males and females. There were no obvious differences in total duration nor in the highest and lowest frequencies between distance calls before and after unilateral lesioning. Acoustic structures of distance calls in males after unilateral RA lesion had upward rapid frequency

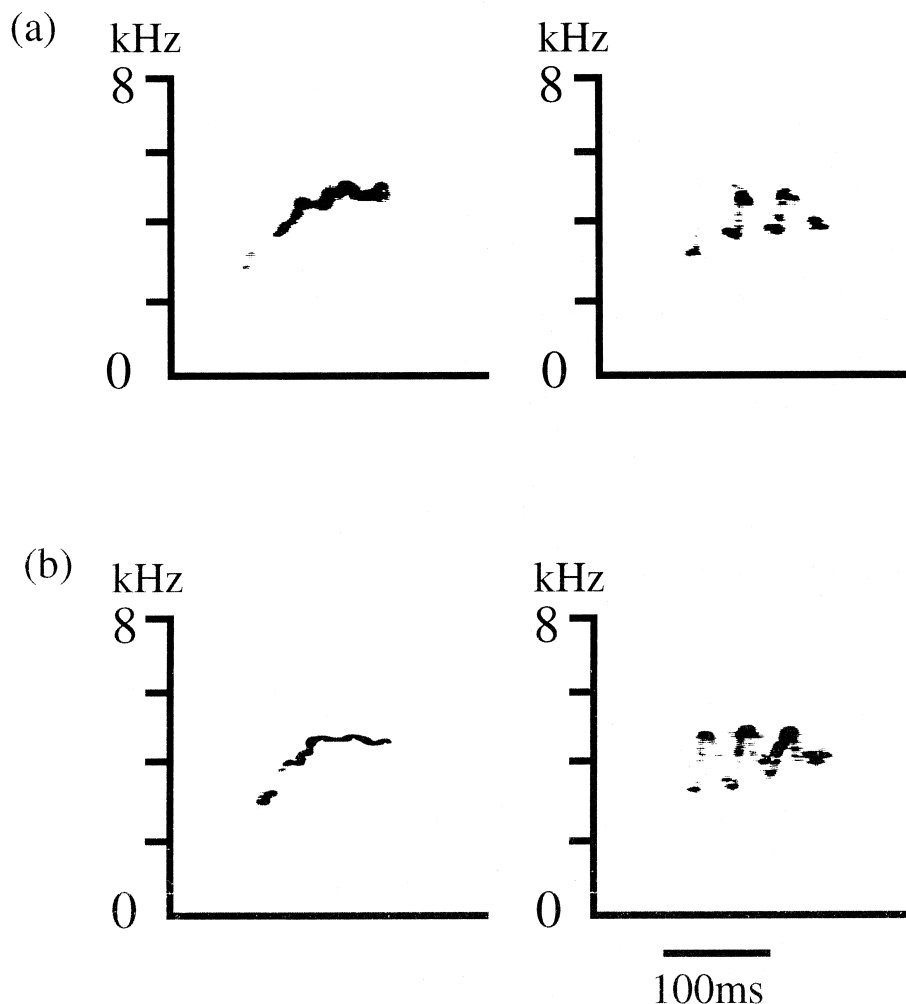


Fig. 6 (a) Sonograms of distance calls before (left) and after (right) bilateral RA lesions in sexually mature male. (b) Sonograms of distance calls before (left) and after (right) unilateral RA lesion in sexually mature male.

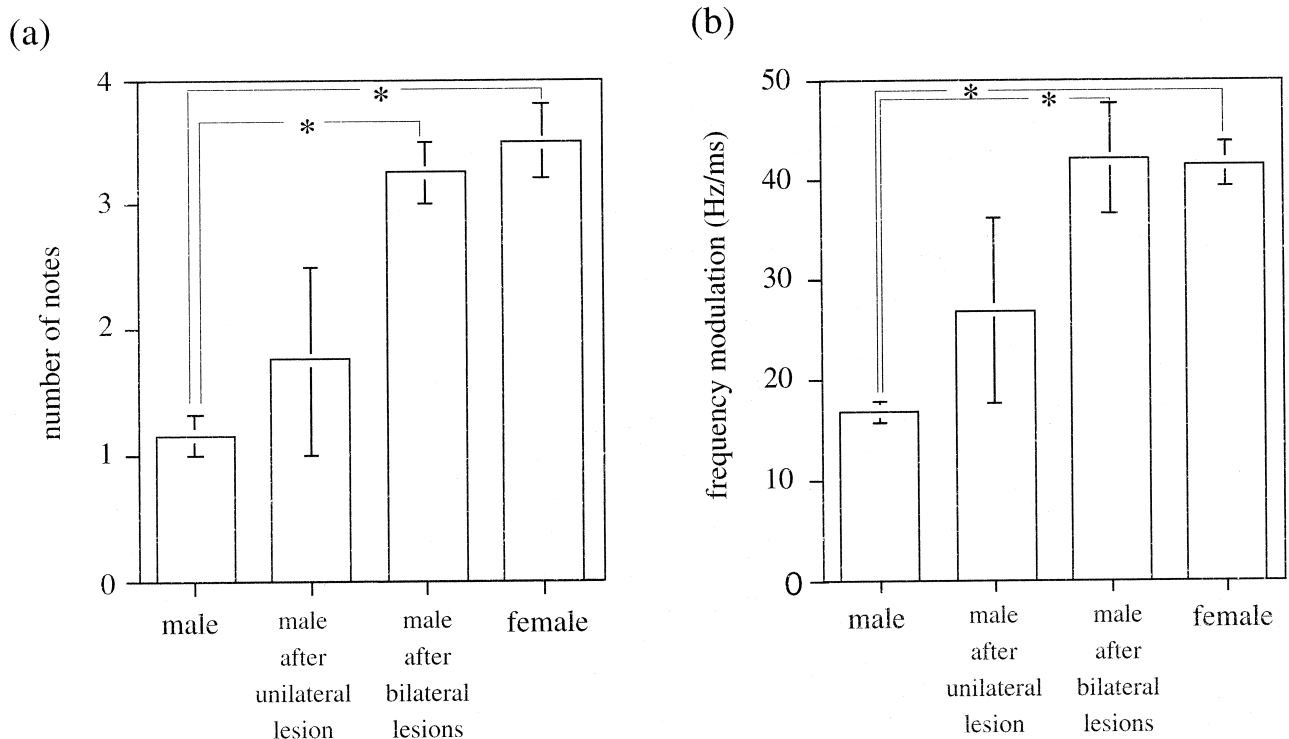


Fig. 7 Representative number of notes (a) and frequency modulation (b) of distance call in intact male, male after unilateral and bilateral RA lesions, and intact female. Error bar indicates S.E.M. Asterisk indicates significant difference (t-test, $p < 0.01$).

modulation as that of female had. Those in male after unilateral RA lesion also had rapid downward frequency modulation. Those in male after unilateral lesion had a few note as that of male. Those in male after unilateral RA lesion was midway between those of typical male and female; it had characteristics which sexually mature male and female had. Distance calls 3 days after unilateral lesioning had partly different acoustic structures (in numbers of notes and the temporal pattern of frequency modulations), but distance calls 7 days after unilateral lesioning showed a more rigid acoustic structure than did those 3 days afterward. Distance calls recorded 7 days and 28 days after unilateral lesion, however, had similar acoustic structures. There was no obvious difference between acoustic structures of distance calls after left or right RA lesioning.

DISCUSSION

Both male and female Bengalese finches produce distance calls. In sexually mature male and female Bengalese finches, electrolytic lesions of the bilateral DM eliminated distance calls and other kinds of calls, and electrical stimulation to one of the DM induced calls that were acoustically similar to male and female distance calls. These results confirm that the DM in Bengalese finches is one of the nuclei of the call control system, as reported in several other avian species (Brown 1965a, Seller, 1980; Vicario and Simpson, 1995).

In sexually mature Bengalese finches, the acoustic structures of distance calls show sexual differences: sexually

mature males sing songs, but sexually mature females do not, as reported in canaries (Nottebohm and Arnold, 1976). This behavioral dichotomy is due to sexual dimorphism in the song control system, as has also been reported in canaries (Nottebohm and Arnold, 1976). The song control system of sexually mature male Bengalese finches resides within the hyperstriatum ventrale pars caudale (HVC) and the RA, but in sexually mature females the HVC and RA are either rudimentary or unrecognized.

To clarify the relationship between this sexual dichotomy in the acoustic structures of distance calls and sex differences in the central motor system that produces distance calls, we investigated the neural pathway from and to the DM in the call control system by means of anatomical tracing. Our results show that the DM in the sexually mature male receives neural input from the RA, but the DM of the sexually mature female does not. Previous papers reported the neural input from RA to DM in male zebra finches (Gurney, 1981; Vicario, 1991; Striedter and Vu, 1998). In sexually mature male and female Bengalese finches, we revealed that fibers from the DM innervated the nXIIIs and ipsilateral DM. Neural connections between bilateral DMs were also observed in zebra finches (Wild, 1997; Striedter and Vu, 1998). These results in Bengalese finches also suggest that neural signals of the distance call descend the pathway from the DM to the contralateral DM and nXIIIs in sexually mature males and females, and neural signals from the contralateral DM regulate neural information in the ipsilateral DM.

We speculated that neural input from the RA to DM of

sexually mature male Bengalese finches modulates the acoustic structure of its distance call. To confirm this idea, we analyzed acoustic structures of distance calls before and after electrolytic lesions of the bilateral RA in sexually mature males. After bilateral RA lesioning, these males did produce distance calls acoustically similar to those of females. In young male and female Bengalese finches, the song control system of the HVc and RA is rudimentary, as reported in zebra finches (Konishi and Akutagawa, 1985), and acoustic structures of distance calls in young male and female Bengalese finches are similar to those of the sexually mature female (Yoneda and Okanoya, 1991). These results suggest that neural input signals from the RA to the DM do indeed produce the differing acoustic structures of distance calls in sexually mature males.

In the mature male Bengalese finch, anatomical tracing of DM and of electrolytic lesions of the bilateral RA show that neural information from the RA to the DM relates to generate the characteristic acoustic structures of distance calls. The physiological function of the DM in producing that distance call, however, is not yet clear. Our ethological results in this report clarified sexual difference of functions of DM. Further work is needed to identify the neural circuits in the DM that serve as the locus for vocal call generation.

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