Control pattern of vocal center for vocalization in ruddy bunting (*Emberiza rutila*)

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Received January 27, 2003

Abstract High vocal center (HVC) can produce single sound with one or two syllables by the single-type vocal control pattern in songbirds ruddy bunting (*Emberiza rutila*). It obviously shows left-side dominance in controlling double syllables, principal frequency (PF) and increasing sound intensity of the evoked calls. Meanwhile, the complex-type control pattern can produce complex calls with multisyllable, and also shows significant left-side dominance in controlling the number of syllables, tone changing and sound intensity. These indicate that left-side HVC controls higher frequency and complicated sentence structure. The basic vocal center, dorsomedial nucleus of the intercollicular complex (DM), controls the monosyllable sound in songbirds, and shows left-side dominance in controlling both the number of syllable and sound intensity. These results not only provide some direct evidence for left-side dominance in high vocal center, but also indicate that there is some internal connection between the high and basic vocal centers in songbirds.

Keywords: ruddy bunting, high vocal center, basic vocal center, control pattern, left-side dominance.

DOI: 10.1360/02yc0079

In recent studies, some progress has been made in the vocal mechanism of songbird by using both electrophysiological and acoustics technique[1—3]. High vocal center (HVC) is the highest center in the vocal control pathway in songbirds.

Unilateral stimulating HVC may evoke bilateral electrical activity of HVC neurons[4,5]. The lesion of HVC critically affects the capacity of singing, and birds could produce only simple calls. In addition, the basic frequency decreases, rapid modulation of frequency is lost and the temporal properties of song change[6,7]. Furthermore, it has been found that left-side HVC plays an important role in singing control in many songbirds[8]. Electrical stimulation of midbrain can evoke calls in both songbirds and non-songbirds[9,10]. However, lesion of bilateral dorsomedial nucleus of the intercollicular complex (DM) results in aphonia of the bird, indicating that DM is the basic vocal center of birds[11]. Ruddy bunting (*Emberiza rutila*) is a typical songbird with complex songs except common simple calls, and most of its songs consist of complicated syllables in the range of

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1500—8000 Hz.

In the present study, we reveal the control pattern of different level vocal nuclei (HVC and DM) in songbirds by electrical stimulation and acoustic analysis methods. The results show that both HVC and DM show left-side dominance in vocal control pattern, providing the acoustic evidence for the vocal mechanism in songbirds.

1 Materials and methods

Seventeen healthy adult male ruddy buntings (RB) were chosen during April to July. Surgery was performed under anesthesia induced by injections of 25% urethane (0.5 g/kg weight). The head of bird was placed on a stereotaxic apparatus. The stimulating nuclei were placed stereotaxically according to modified coordinates from the canary brain atlas by Stokes[12]. The electrode (pointed end diameter, 80 m) was injected into the certain brain nuclei as follows. The coordinates of HVC and DM are AP: 0.0—0.6, L/R: 1.2—2.8, H: 0.2—1.0 and AP: 1.1—1.2, L/R: 2.0—2.5, H: 5.3—5.7, respectively. The electric stimulating pulse was produced by a stimulator with a wave width of 0.5 ms and frequency between 10 and 80 Hz. The evoked calls were recorded after they became stable. Perfusion was performed in 100 mL saline and 4% formaldehydum polymerisatum for 30 min, and then the brain was completely removed from the skull. After overnight immersion in 30% sucrose PBS, the brain was frozen-sectioned at 40 μm, stained and examined under a microscope.

The evoked calls were recorded by a tape recorder Model SHARP-GF-6060 and inner microphone (with a response frequency of 50—13000 Hz), at a distance of 20 cm from the sound source. Recorded calling sentences were orderly examined by a sonagraph Model 662B (with frequency response of 85—12000 Hz and filter with bandwidth of 45 Hz). The response frequency selection is high frequency rising (raising 6 dB per octave from 1 kHz). The temporal properties, waveform and spectrum were further measured and analyzed by MATLAB software.

2 Main results

2.1 Vocal control pattern of HVC in ruddy bunting

As shown in fig. 1, HVC has a single-type control pattern for the evoked calls during stimulation. The evoked calls are smooth single syllables. Evoked calls from left-side are similar to “jue-jue • jue-”. As shown in fig. 1(a), two monosyllables ($SS_{1,2}$) consist of repeated calls followed by a single call with one monosyllable (SS_3). The sonogram of monosyllable is almost straight and smooth, and the sound length and main energy frequency are 120—310 ms and about 4800 Hz, respectively. The corresponding spectrum is shown in fig. 1(c). The principal frequency (PF) is 4800 Hz. The amplitude of PF is 16.5 mV and the bandwidth at level 10 dB below PF is about 540 Hz. The average PF in different samples is 5190 ± 420 Hz (10). The coefficient of variation (SD/average × 100%) is only 8.1%. Therefore, there is no obvious difference between the two kinds of monosyllable. They are both smooth monotone sounds with PF as the dominant sound.

The evoked calls from right-side HVC of RB, sound like "jue- • jue" as shown in fig. 1(b). The single call consists of only one monosyllable ($SS_{1,\ 2}$), and its sonogram is almost smooth. The sound duration is 200—210 ms, and PF is about 4200 Hz. In fig. 1(d), the corresponding spectrum shows that PF is 4050 Hz, and the amplitude of PF is 5.4 mV, the bandwidth at level 10 dB below PF is about 360 Hz. Two lower amplitude humorous frequencies (f_1 and f_2) around 2000 and 6000 Hz can be observed. Their amplitude is about 33 dB lower compared with PF. The average PF is 4575±180 Hz (4) in different samples; the coefficient of variation is only 3.9%. So it is similar to the left side, that is, there is no obvious difference among the monosyllables. They are all smooth monosyllables with PF as the dominant sound. Compared with the evoked calls from left-side, those of right-side are all repeated monosyllables, and the PF has significantly decreased by 2.2 semitone (39.86 log (4575 Hz/5190 Hz)) (t-test, t = 2.772>$t_{0.05}$, d.f. (Degree of freedom) =12). Meanwhile, the amplitude of PF and bandwidth of main energy band are decreased by 9.7 dB and 33.3%, respectively.

It can be seen that the single-type vocal control pattern of HVC in RB shows significant left-side dominance in double syllables, properties of PF and sound intensity increasing of evoked calls.

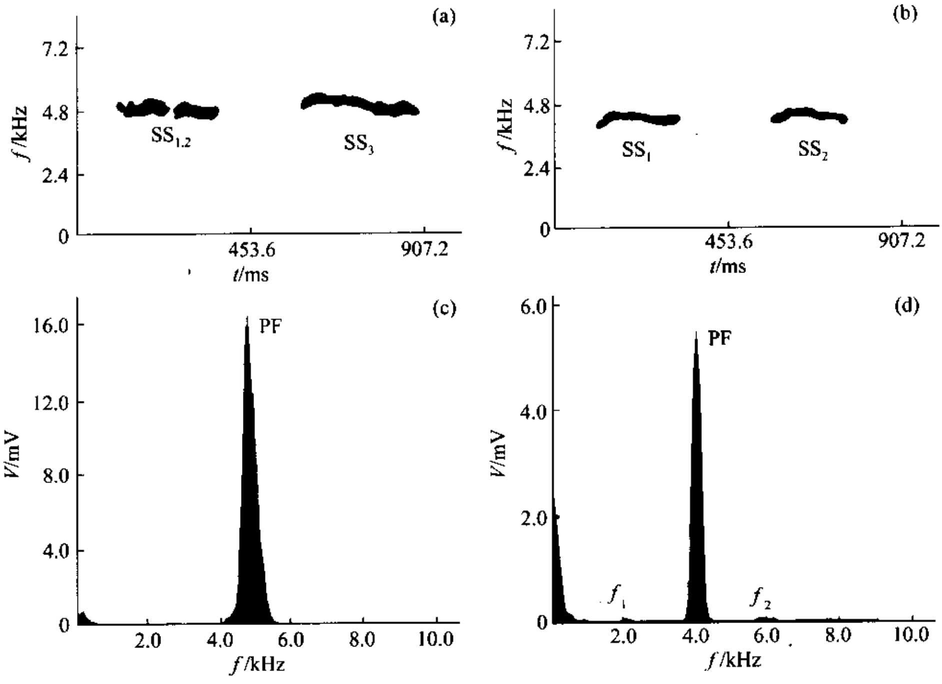

Fig. 1. Sonograms and spectra of single type control pattern from HVC in ruddy bunting. (a) and (c), (b) and (d) show the evoked calls from left- and right-side, respectively. (a) and (b) are sonograms. SS_{1-3} are monosyllables. (c) and (d) are spectra. PF is principal frequency. $f_{1,2}$ are components of low amplitude frequency.

Some individuals can produce multisyllable sounds evoked by the complex-type vocal control pattern under the same condition, as shown in fig. 2. The multisyllable ($MS_{1—3}$) calls from left-side HVC contain 8, 3 and 5 syllables, respectively. And the repeated cycles are 25.5, 32.4 and 40.5 ms, the shorter sound length is about 8 ms, and the longer sound length is about 32 ms. Meanwhile, the high and low frequencies are about 5800 Hz and 4300 Hz, respectively. Some syllables (such as 1, 2, 3 and 5 syllable in MS_3) exhibit a little drop of tune, that is, the sonogram of syllables changes gradually from high to low frequency. In the corresponding spectrum (fig. 2(c)), PF is 4700 Hz and the main energy bandwidth is about 850 Hz when PF decreases by 10 dB. Meanwhile, the right of the spectrum has an obvious change of tone decreasing, and the amplitude of three frequency components ($f_{1—3}$ are about 4380 Hz, 5760 Hz and 6350 Hz, respectively) are 3.4, 18.5 and 20.7 dB lower than those of PF (5.9 mV), that means the decreasing rate is about 32—138 Hz /dB. It can be concluded that the multisyllable calls evoked by the complex-type vocal control pattern from left-side HVC are monotone sounds with PF as the dominant sound, and have an obvious change of tone decreasing.

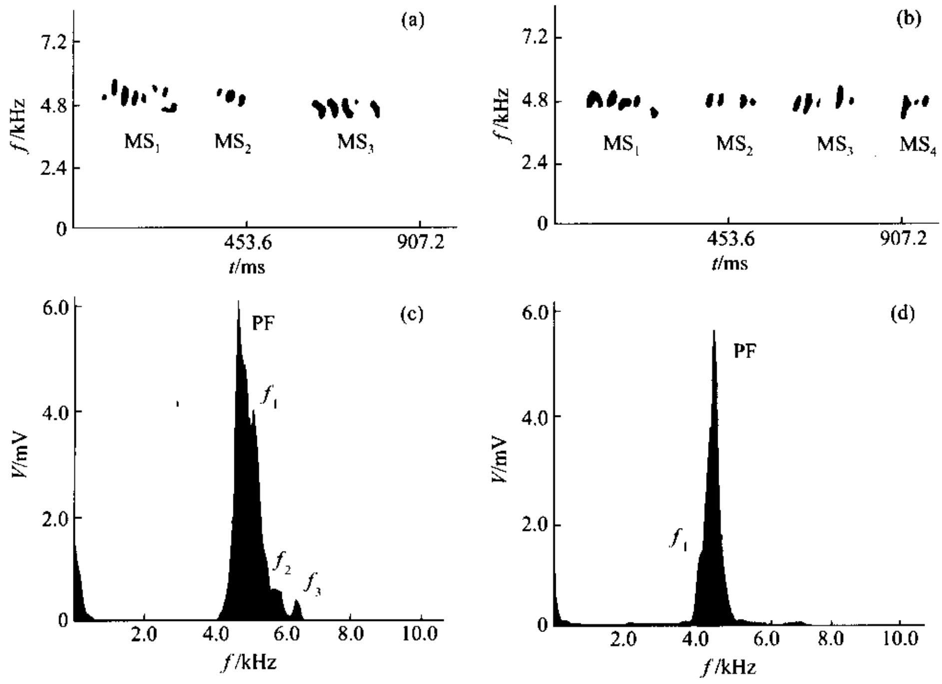

Fig. 2. Sonograms and spectra of complex-type control pattern from HVC in ruddy bunting. (a) and (c), (b) and (d) show the evoked calls from left- and right-side, respectively. (a) and (b) are sonograms. $MS_{1—4}$ are multisyllables. (c) and (d) are spectra. PF is principal frequency. $f_{1—3}$ are the other frequency components.

The multisyllable ($MS_{1—4}$) calls from right-side HVC contain 5, 4, 5 and 3 syllables, respectively. The repeated cycles are 42.2, 41.0, 38.4 and 30.8 ms; the shorter sound length is about 10 ms; the longer sound length is about 40 ms. Meanwhile, the high and low frequencies are about

5800 Hz and 4300 Hz, respectively. However, some syllables (such as 1, 2 and 4 syllable in MS_3) exhibit a small increase of tune, that is, the sonogram of syllables changes from low to high frequency. In the corresponding spectrum (fig. 2(d)), PF is 4500 Hz and the main energy bandwidth is about 480 Hz when PF decreases 10 dB in the corresponding spectrum (fig. 2(d)). Simultaneously, the left in the spectrum has a little tone increasing, and the amplitude of one component frequency (f_1 of about 4380 Hz) is 11.3 dB lower than that of PF (5.4 mV), that means the increasing rate of time is only 11 Hz /dB. It indicates that the multisyllable sounds, evoked by the complex-type vocal control pattern from right-side HVC, are monotone sounds with PF as the dominant sound, and have a slight tone rise.

It can be seen that the multisyllable calls are mainly composed of PF evoked from bilateral HVC by complex-type vocal control pattern in RB. Moreover, the syllables number, tone changing rate and the bandwidth of main energy from left-side are about 1.3, 2.9 to 12.5, and 1.8 times higher, as compared with those of right-side HVC, respectively. It also shows significant left-side dominance.

2.2 Vocal control pattern of DM in ruddy bunting

The evoked calls from both left- and right-side DM are similar to “ga • ga ...” in RB. There is obvious difference in the single calls evoked form left-side DM in sonogram, as shown in fig. 3(a). One of them sounding like “ga” is monosyllable (S_1) and its sound length is about 40 ms. Some of them have two syllables ($S_{2,3}$), consisting of basic sound (BS) and one upper partial (UP), with sound length of about 80 ms. The frequency range of BS and UP is 3000—7500 Hz and 9500—12000 Hz, respectively. In the corresponding spectrum (fig. 3(c)), the PF of BS is 5070 Hz, and the range of frequency is from 4000 to 7000 Hz when the amplitude of PFs is decreased by almost 70% (equal to decreasing 10 dB). The bandwidth of main energy band is about 3000 Hz. In sonogram, the energy of UP is rather low, so it cannot be seen clearly in the spectrum. The evoked calls are monosyllables with PF as the dominant sound.

The sonogram of evoked calls from right-side DM is similar to that of left-side. Each single call sounding like “ga” has a syllable (S_{1-3}), and its sound length is about 60 ms, and all calls are composed of BS and one UP, the frequency range of which is 3000—7000 Hz and 9000—12000 Hz. In the corresponding spectrum (fig. 3(d)), PF of BS is 5300 Hz, and the frequency range is about 4700—6000 Hz when the amplitude of PFs (~7.2 mV) is decreased by 10 dB. It means that the bandwidth of main energy band is ~1300 Hz. These results are similar to that of the left side, that is, energy of UP is very low in sonogram and could not be detected in spectrum. The evoked calls are monotones with PF as the dominant sound.

These data suggest that there is no obvious difference in the timbre properties of single evoked calls from both the left- and right-side DM, because they are all monotone calls with PF as the dominant sound and have similar PF. However, the amplitude of PF and bandwidth of main energy band in single calls, mono-structure syllables from right-side DM, decrease by 27.2 dB and

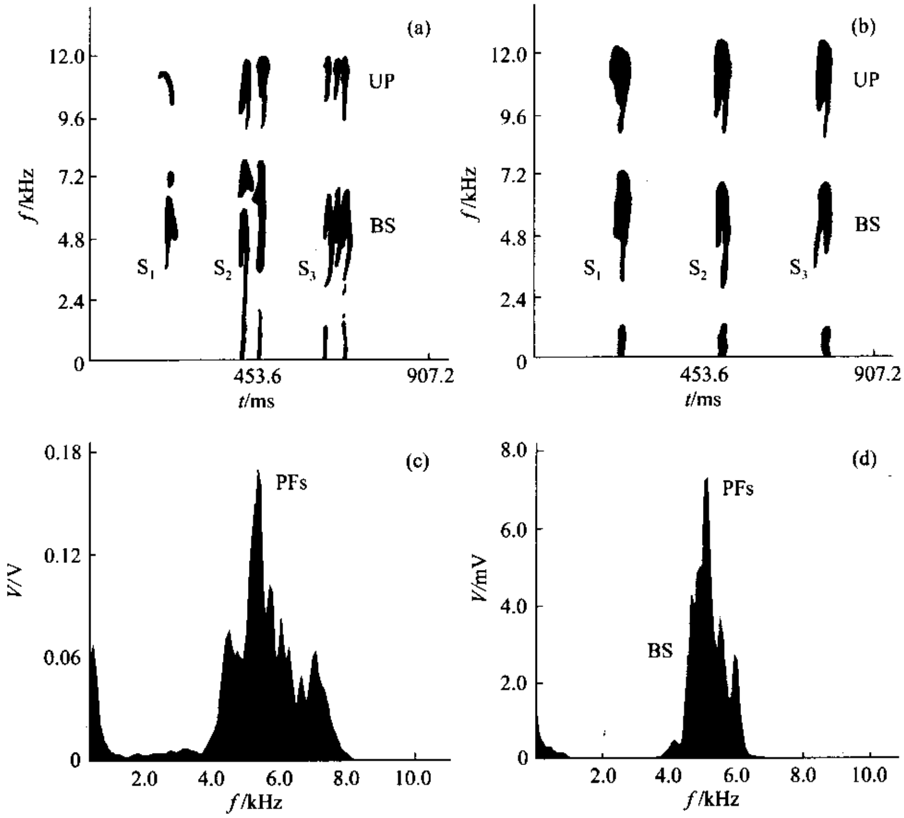

Fig. 3. Sonograms and spectra of evoked calls from DM in ruddy bunting. (a) and (c), (b) and (d) show the evoked calls from left- and right-side DM, respectively. (a) and (b) are sonograms. S_{1-3} are single calls "ga"; BS is basic sound; UP is upper partial. (c) and (d) are spectra. BS is basic sound; PFs is principal frequency of BS.

1700 Hz, respectively, compared to those of left-side. It shows that the basic vocal center (DM) is significantly left-side dominant in the syllable number, frequency range of main energy band and properties of sound intensity from the evoked calls in RB.

3 Discussions

High vocal center (HVC) projects to nucleus robustus archistriatalis (RA) involving vocal control pathway, and also projects to X area to take part in vocal learning pathway [13,14]. Different neurons have different reactions to sound signals, but all of them can generate action potentials and participate in the process of auditory feedback[15]. DM receives the projections directly from forebrain RA and sends out fibers directly to nucleus hypoglossi, par tracheosyringealis (nXIIts) to innervate syringeal muscles[16]. With different degree shrinking of syringeal muscles, songbirds are able to produce various songs[17,18]. There are neural connections between bilateral DM[10]. In natural situation, there is an auditory feedback process of perception, modulation and correction to their own songs during vocal learning in songbirds[19,20].

The syllables in natural songs are abundant and various, with wide frequency-changing range, concentrated energy and high loudness. However, after lesion of HVC, high frequency syllables are losing, energy is lower, songs are flat and the loudness decreases greatly. Furthermore, the remaining syllables are different, resulting from lesion of left- and right-side HVC. The effect of left side lesion has more severity than that of right side lesion. That indicates left-side dominance in HVC during vocal control. HVC influences syllable properties, such as time length, frequency and energy, and the frequency changing and energy concentration rely on intact HVC. In the present study, left-side dominance is shown not only in monosyllable sounds with sentence structure, PF and amplitude, but also in multisyllable sounds with syllable number and tone changing from the evoked calls while stimulating bilateral HVC. It suggests that left-side HVC controls higher frequency, more complicated sentence structure, syllables and tone changing as well. The innervation of syringeal muscles has unilateral character for nerve hypoglossi, pars tracheosyringealis (NXIIts). Bilateral syrinx can produce calls independently, and it also shows left-side dominance in acoustics features of the syllable[1,21]. These results are similar to the lateralization in the evoked calls from HVC.

DM is considered as the basic vocal center, because it can produce simple calls similar to frightened calls rather than songs in both songbirds and non-songbirds. Moreover, the stimulating threshold is the lowest in the midbrain vocal center[10]. The control pattern of DM shows obvious left-side dominance in syllable number and sound intensity modulation in RB.

The difference in the vocal control pattern between HVC and DM could be explained by that HVC is located in a higher position, and that DM is lower in the vocal control pathway. The evoked calls from stimulating the two nuclei, HVC and DM, show left-side dominance in acoustics properties. It indicates that there is an internal connection between high and basic vocal centers in songbirds.

While the evoked calls were recorded, some other body movements, such as drooling, mydriasis, defecation, faster respiratory frequency and vegetative activities were observed during stimulating HVC and DM. It is the obvious facilitated effect. It indicates HVC and DM are involved in the modulation of respiration[22,23]. Recent data reveal that DM plays an important role in the respiration movement, and DM receives the descending projections from RA, and then influences respiratory-vocal process by sending out fibers to medulla oblongata ventral intermediate nucleus where the inspiratory premotor nucleus is located[24—26]. But there are still some unclear mechanisms of the complicated projecting pathway and respiration-vocal regulation. These would be meaningful topics for further research.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 39570195), Outstanding Youth Foundation of Ministry of Education of China, Science and Technology Research Project of Ministry of Education of China (Grant No. 01062) and Laboratory of Visual Information Processing Foundation of the Chinese Academy of Sciences.

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