

Role of Syringeal Muscles in Controlling the Phonology of Bird Song

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SUMMARY AND CONCLUSIONS

1. The contribution of syringeal muscles to controlling the phonology of song was studied by recording bilateral airflow, subsyringeal air sac pressure, electromyograms (EMGs) of six syringeal muscles, and vocal output in spontaneously singing brown thrashers (*Toxostoma rufum*).

2. EMG activity in musculus syringealis ventralis (vS), the largest syringeal muscle, increases exponentially with the fundamental frequency of the ipsilaterally generated sound and closely parallels frequency modulation.

3. The EMG activity of other syringeal muscles is also positively correlated with sound frequency, but the amplitude of their EMGs changes only a small amount compared with variation in the amplitude of their EMGs correlated with changing syringeal resistance. The elevated activity in all syringeal muscles during high-frequency sounds may reflect an increased need for structural stability during the strong contractions of the largest syringeal muscle (vS).

4. Several syringeal mechanisms are used to generate amplitude modulation (AM). The most common of these involves modulating the rate of syringeal airflow, through activity by adductor (m. syringealis dorsalis and m. tracheobronchialis dorsalis) and abductor (m. tracheobronchialis ventralis) muscles, which change syringeal resistance, switch sound production from one side of the syrinx to the other, or produce rapid oscillatory flow changes. Variation in the phase relationship between AM and EMG bursts during oscillatory airflow suggests complex biomechanical interaction between antagonistic muscles.

5. AM can also arise from acoustic interactions of two independently generated sounds (beat notes) including cross talk signals between the two syringeal halves. In this latter mechanism, sound generated on one side radiates slightly out of phase with the source from the contralateral side, resulting in lateralized AM generation.

INTRODUCTION

Song production in oscine birds is a complex learned behavior and its neural control is correspondingly complex. Despite extensive study (e.g., Margoliash et al. 1994; McCasland 1987; Nottebohm 1991; Vicario 1991a; Vu et al. 1994; Williams and Vicario 1993), many details about the organization of control and specific control mechanisms are unknown. One reason for this is that the expression of the central control patterns at the level of the syrinx as well as the biomechanical events leading to sound generation are not understood adequately. The experiments reported here attempt to elucidate the neuromotor mechanisms of this important form of vocal communication.

A number of brain nuclei whose interconnections are well characterized in songbirds form a network that controls singing behavior (e.g., Konishi 1985, 1989; Nottebohm et al. 1976; Vicario 1991a). Central song centers must coordinate

different neuromotor systems that contribute to sound generation, such as respiratory musculature (Hartley 1990; Vicario 1993; Wild 1993), syringeal musculature (Goller and Suthers 1995, 1996; Vicario 1991b), and muscles controlling beak and neck movements (Hausberger et al. 1991; Nowicki 1987; Westneat et al. 1993).

The syringeal muscles, innervated by the ipsilateral tracheosyringeal branch of the hypoglossal nerve, act on structures of the vocal organ, the syrinx, and their functions are essential for proper sound production (e.g., Miskimen 1951; Nottebohm 1968). We attempted to investigate the role of individual syringeal muscles during sound production in brown thrashers (*Toxostoma rufum*) by simultaneously measuring bronchial airflow, subsyringeal air sac pressure, and electromyographic (EMG) activity in the syringeal muscles.

Sound is presumably generated when air flowing through the syrinx sets the medial tympaniform membranes into vibration. The rate of syringeal airflow depends on the subsyringeal air sac pressure and changes in the aperture of the syrinx that determine its resistance to airflow (Brackenbury 1989; Fletcher 1988; Gaunt 1987). Syringeal resistance is thought to be controlled by adductive and abductive action of syringeal muscles that turn sound production on or off (i.e., gate sound production) by rotating the lateral labium into or out of the syringeal lumen (Chamberlain et al. 1968). EMG recordings largely confirm the proposed gating activity of the syringeal muscles. Activity in the dorsal muscles, m. syringealis dorsalis (dS) and m. tracheobronchialis dorsalis (dTB), coincides with periods of active adduction, suggesting that these two muscles are the main adductors. The main antagonist abducting the syrinx is m. tracheobronchialis ventralis (vTB) (Goller and Suthers 1995, 1996).

In addition to gating functions, syringeal muscles are thought to influence or control the phonetic structure, frequency modulation (FM), and amplitude modulation (AM) of the generated sound. Sound frequency is assumed to depend on the tension of the sound-generating membranes and to be actively regulated by action of syringeal muscles (Brackenbury 1989; Gaunt 1987). Sound intensity is determined by the activity of the respiratory muscles and is modified by the activity of the syringeal muscles and muscles affecting the acoustic properties of the vocal tract (Nowicki 1987; Westneat et al. 1993).

Here we attempt to identify the salient contributions from different syringeal muscles to the phonetic structure of the vocalizations by correlating their electrical activity with properties of the sound, respiratory dynamics, and syringeal biomechanics. We show that specific patterns of muscle activation are associated with changes in the phonology of the

vocalizations, indicating precise neuromotor control over this complex behavior.

METHODS

Nine adult male brown thrashers were used in this study. The procedures used to record and analyze bilateral airflow, sub-syringeal air sac pressure, and EMGs from syringeal muscles in brown thrashers (*T. rufum*) have been previously described in detail (Goller and Suthers 1996; Suthers et al. 1994), so only the main points will be repeated here.

Preparation and surgical implantation

Before surgical implantation of recording devices, 1,500–2,000 syllables of song were recorded from each individual. Singing frequency was enhanced by implantation of testosterone propionate pellets (15 mg sc). A silastic cannula was inserted into the anterior thoracic air sac under isoflurane anaesthesia delivered in breathing air. The cannula was attached to a piezoresistive pressure transducer mounted on a self-gripping fastener (Velcro) on the back of an elastic belt around the bird's thorax. One or 2 days later birds were anesthetized with chloropent (4 μ l/g im) and the syrinx was exposed through an incision in the skin and the interclavicular air sac between the clavicles. The rate of airflow through each side of the syrinx was measured with a heated microbead thermistor inserted into the lumen of each primary bronchus four rings caudad from the syrinx and secured in place with tissue adhesive (cyanoacrylate; Nexaband, Veterinary Products Laboratories). Up to four pairs of bipolar EMG electrodes (0.025-mm stainless steel wire insulated except of the tip, California Fine Wire) were inserted into syringeal muscles (Fig. 1) and secured to the outermost fascia with tissue cement. Wires were led out of the air sac and routed subcutaneously to the back. The air sac was then closed with surgical sutures and tissue cement. Electrical connections of flow probes and EMG electrodes to signal conditioning and recording instrumentation were established through a microconnector attached to the Velcro tab on the back.

Recording

Between 1 and 3 days after surgery, thrashers started to sing again and up to eight channels (vocalization, bilateral airflow, air sac pressure, and EMGs from 2–4 syringeal muscles) were recorded on a rotary storage recorder (RSR 512, Metrum Information Storage). The vocalizations were recorded with a microphone in front of the cage. The rate of airflow through each side of the syrinx was determined as the amount of electrical current required to keep the bronchial thermistors at a constant temperature (Hector Engineering). Air sac pressure measurements were used to determine the direction of airflow. Electrical signals from the syringeal muscles were filtered (band pass 200–3000 Hz) and differentially amplified (Princeton Applied Research, Model 113).

EMG recordings were obtained from six different syringeal muscles (Fig. 1b). We recorded from each extrinsic muscle, tracheolateralis (TL) and m. sternotrachealis (ST) in two birds and from each intrinsic muscle in at least three birds, with the exception of dTB, for which we obtained only one recording. Access to dorsal muscles, dS and dTB, was limited to the lateral portion. In TL we recorded only from the ventral subunit. M. syringealis ventralis (vS) consists of a medial part that is exposed and a lateral part that underlies vTB. We recorded only from the medial part (Fig. 1).

Data analysis

All parameters were digitized (Data Translation, DT 2821-G) and subsequently analyzed with the use of Signal software (Engi-

neering Design, v. 2.3). Data on the rate of airflow are presented in nonlinear, relative units. Because the bronchial wall is not rigid, the dimension of the lumen may change during song, so the output of the thermistor could not be calibrated for an absolute flow rate.

Up to ~4 kHz, the thermistors also responded to air movement associated with near field sound in the bronchus. High-pass filtered (>300 Hz) flow recordings thus revealed the fundamental frequency (f_1) of the ipsilaterally generated sound (Suthers et al. 1994). These recordings of bronchial sound facilitated determination of the f_1 and confirmed the respective contributions of the left and right side of the syrinx. EMG recordings were rectified (Signal ENV procedure, time constant 0.1 ms) for quantification of electrical activity and better visualization.

RESULTS

Fundamental frequency

Vocalizations with low and constant f_1 are associated with weak activation in all ipsilateral syringeal muscles. Syllables with higher f_1 are generally accompanied by higher-amplitude EMGs (Fig. 2). However, these proportional relationships are different for different syringeal muscles.

EMG ACTIVITY IN vS. EMG activity in vS closely reflects the f_1 of the ipsilaterally generated sound (Figs. 2–4). Examples from two thrashers, showing patterns of ipsilateral vS EMG activity for frequency components of syllables generated on the left (Fig. 3) and right side (Fig. 4) of the syrinx, illustrate the consistent correlation of f_1 and vS activity. The amplitude of EMG activity in vS rises exponentially with increasing f_1 of ipsilaterally produced syllables (Fig. 5) and encompasses the entire range of vS EMG amplitudes encountered. This close relationship between f_1 and EMG amplitude was found without exception for all vS recordings from different individuals and left and right sides (Table 1). However, the coefficients of determination are lower for the right side (average $R^2 = 0.76$, $N = 3$) than for the left side (average $R^2 = 0.89$, $N = 4$; test of homogeneity, $\chi^2 = 55.85$, $P < 0.001$) (Sokal and Rohlf 1995).

Some syllables with oscillatory flow changes and AM are accompanied by vS EMG amplitudes higher than expected from the otherwise close correlation between frequency and EMG amplitudes. Such syllables account for most of the variability above the regression lines in Fig. 5. During syllables with oscillatory changes in airflow, vS activity can vary from sustained EMG activity to bursts synchronized with FM and AM (Goller and Suthers 1996). The highest amplitudes of bursts in vS were found in syllables with marked activity in all syringeal muscles (see below). This may explain why correlations of vS activity and f_1 are not as strong for right vS as for left vS, because syllables with rapid flow modulations are more frequently produced on the right side (Suthers et al. 1994).

FM. EMG activity in vS also changes during pronounced FM, such that upward FM is accompanied by increasing EMG strength and downward FM by reduced EMG activity (Fig. 6). Even small changes in sound frequency are reflected in the activity patterns of vS. Thus the EMG pattern in vS predicts the occurrence of FM. The EMG amplitude in other syringeal muscles is not highly correlated with FM. **FREQUENCY IS ONLY WEAKLY CORRELATED WITH SYRINGEAL RESISTANCE.** To test whether the close relationship of vS activity and f_1 might be attributable to covariation of fre-

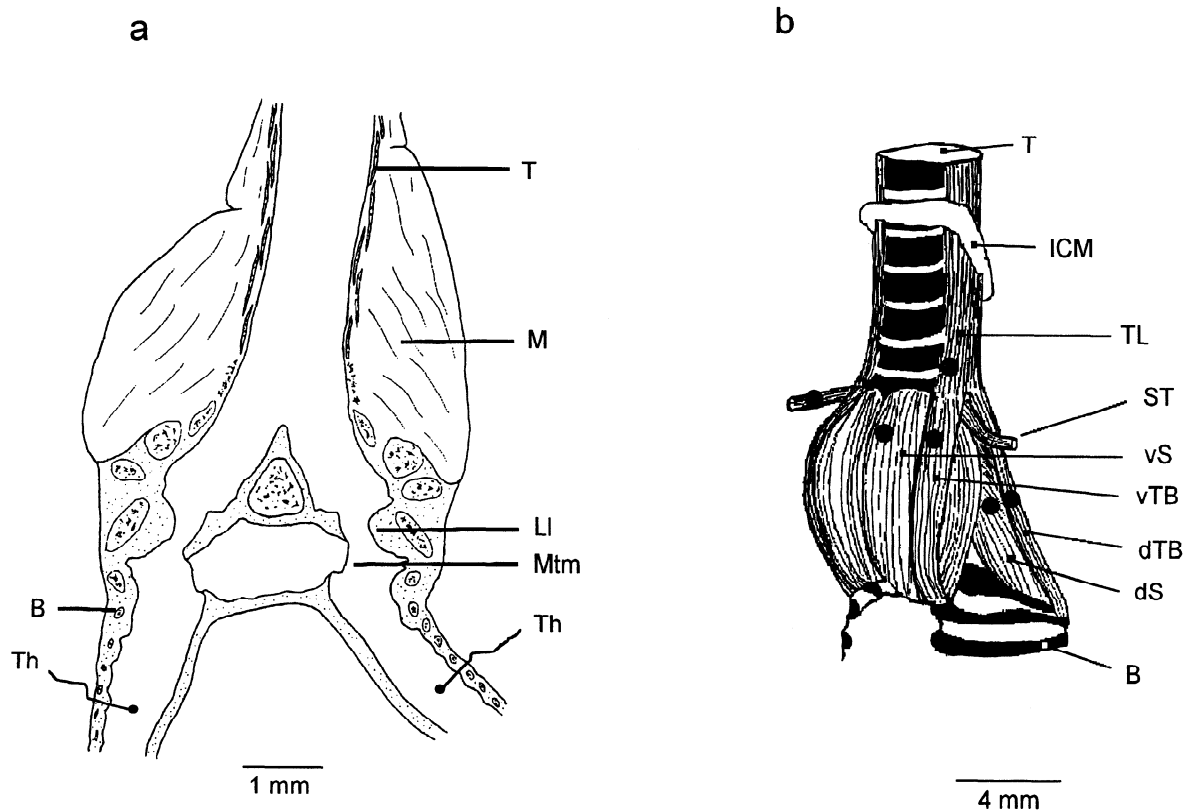


FIG. 1. *a*: frontal section of a thrasher syrinx depicting the main morphological structures and the positioning of thermistors for airflow recordings. The medial tympaniform membranes (Mtm) are believed to be the sound-generating structures. Airflow on each side of the syrinx is controlled independently through active control of the aperture, presumably by movement of the lateral labium (Li) into (adduction) or out (abduction) of the bronchial lumen (Chamberlain et al. 1968). T, trachea; M, syringeal muscles; Th, thermistor; B, bronchial ring (modified after Suthers 1990). *b*: schematic ventrolateral view of the thrasher syrinx depicting syringeal muscles. Black dots: approximate location of electrode insertion on 1 side. Electrodes were positioned at approximately the same locations on the other side. ICM, membrane of the interclavicular air sac; TL, m. tracheolateralis; ST, m. sternotrachealis; vS, m. syringealis ventralis; vTB, m. tracheobronchialis ventralis [this was found to be the main abductor muscle, i.e., its activity decreases syringeal resistance (Goller and Suthers 1996)]; dTB, m. tracheobronchialis dorsalis; dS, m. syringealis dorsalis [dorsal muscles are adductors, i.e., activity increases syringeal resistance to airflow (Goller and Suthers 1996)] (modified after Goller and Suthers 1995, 1996).

quency with syringeal resistance, correlation coefficients for these two parameters were computed. A positive correlation between the two parameters indicates that the production of sounds with a high f_1 is generally accompanied by elevated ipsilateral syringeal resistance (average $R^2 = 0.22$; Table 2). In a partial correlation analysis in which syringeal resistance is kept constant (Sokal and Rohlf 1995), partial correlation coefficients for vS EMG activity and f_1 still range from 0.92 to 0.85, excluding the possibility that the close association is merely caused by a relationship between resistance and sound frequency. This agrees with our earlier findings that in brown thrashers vS is not directly involved in controlling syringeal resistance (Goller and Suthers 1996).

EMG ACTIVITY IN vTB AND TL. EMG strength in vTB and TL does not reflect f_1 as consistently as that of vS (Figs. 2 and 7). The close correlation between frequency and EMG strength in vTB in the syllables shown in Fig. 2 is not always present. For example, EMG amplitude does not increase despite a 0.5-kHz rise in f_1 in syllable 5 of Fig. 7. The positive correlation between EMG strength and f_1 for vTB and TL is weaker than in vS but is still high (Table 1).

However, the amplitude of vTB EMGs during vocaliza-

tion is typically much less than that of EMG bursts recorded from this muscle during the onset of inspiratory (Fig. 2, TH41), or occasionally expiratory (Fig. 7, TH22), airflow. The changes in EMG amplitude of vTB and TL associated with frequency shifts encompass only a small portion of the full range of EMG activity in these muscles.

EMG ACTIVITY IN ST. EMG activity in ST is not closely correlated with f_1 , but more data are needed to explain the large variability between the two ST data sets (Fig. 4; Table 1). It is difficult to obtain good-quality recordings from this small muscle (Goller and Suthers 1996).

EMG ACTIVITY IN DORSAL MUSCLES. Activity of dS and dTB is strongest during ipsilateral adduction (Figs. 2 and 3). This is consistent with the important role these muscles play in gating sound production through syringeal adduction, as reported in Goller and Suthers (1996). Changes in EMG amplitude with increased f_1 are small and inconsistent compared with those related to adductive action (Figs. 2 and 3). Coefficients of determination of EMG amplitude and f_1 are lowest in dorsal muscles (average $R^2 = 0.26$, Table 1) and drop to an average R^2 of 0.11 after a partial correlation

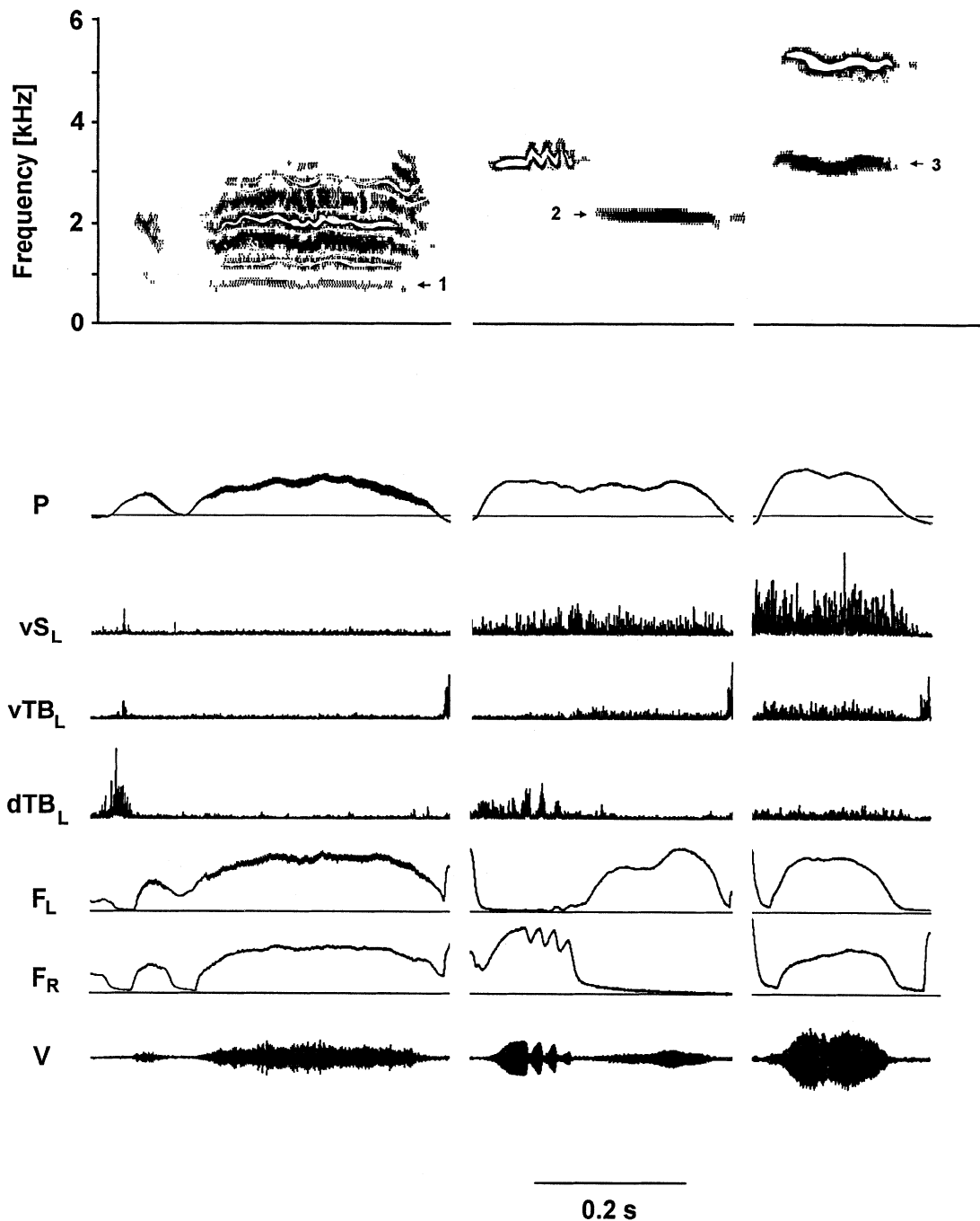


FIG. 2. Electromyographic (EMG) activity in syringeal muscles increases as fundamental frequency (f_1) of sound rises. Three constant frequency syllables of different f_1 (numbered 1–3) sung by *TH41*, and accompanying airflow, air sac pressure, and EMG measurements. Sound is represented oscillographically (V) and spectrographically (*top panel*); right-side-generated sound is marked by outlines on the spectrogram. EMG recordings are rectified (time constant 0.1 ms). Note that EMG activity in dTB is highest during full ipsilateral adduction (i.e., left airflow is 0 despite high positive air sac pressure). The dip in rate of left airflow that occurs ~ 0.1 s after the 1st onset of sound in the 1st syllable is caused by a drop in subsyringeal pressure and is not accompanied by strong dTB_L activity. F, airflow; P, air sac pressure; subscript L, left side of the syrinx; subscript R, right side of the syrinx. Horizontal lines: 0 pressure and airflow.

analysis in which syringeal resistance is held constant (Sokal and Rohlf 1995).

Amplitude modulation

AM RELATED TO SYRINGEAL AIRFLOW. Syringeal muscles can modulate sound intensity by various mechanisms. One

of these is by switching between unilateral and bilateral syringeal airflow, resulting in sound being generated on one or both sides of the syrinx. The first syllable of Fig. 8 consists of three portions of high, low, and intermediate amplitude, respectively. At the beginning of the syllable, amplitude increases with increasing airflow through the left syrinx until

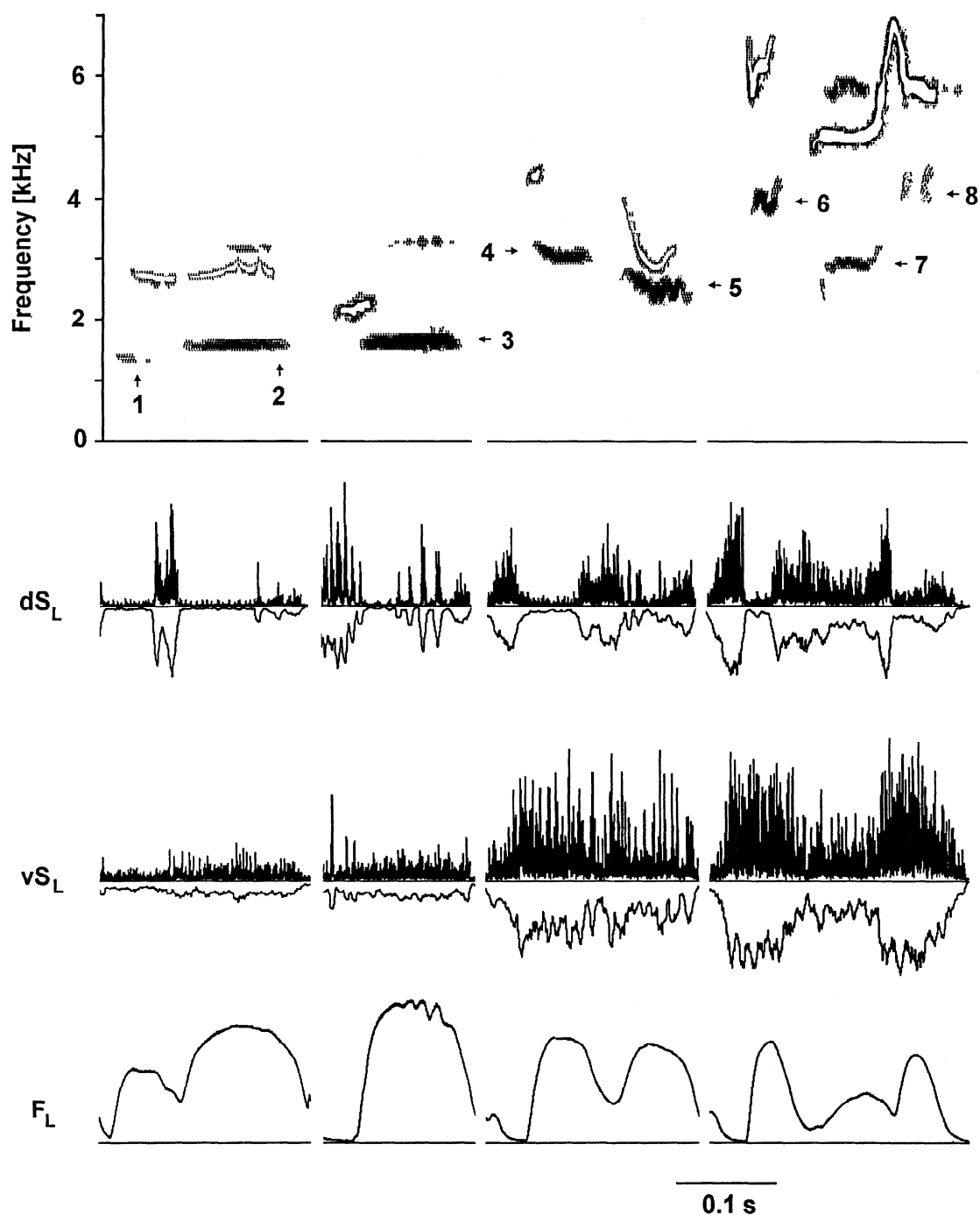


FIG. 3. Song segments shown together with accompanying airflow and EMG measurements in dS_L and vS_L on the left side, illustrating different correlations of EMG activity and f_1 for different muscles. Simultaneously produced right side sounds are outlined. EMG recordings are rectified (upward deflection) and integrated (downward deflection; time constant 1 ms). Abbreviations as in Figs. 1 and 2.

a burst of activity in the left dTB (dTB_L) partially closes the left syrinx, reducing the rate of airflow and the sound intensity. It is likely that the left side is momentarily closed at the end of the first burst of dTB_L activity, but that because of the thermistor time constant left side airflow (F_L) does not drop to zero. F_L begins to increase again as the left syrinx opens after the end of the first burst of activity in dTB_L , but the flow rate remains modest and is soon terminated by left syringeal adduction brought about by a second burst of

activity in dTB_L . The accompanying sound is of low intensity. The final portion of the syllable is produced as the left syrinx again opens (dTB_L activity is low). The abrupt increase in the intensity envelope (Fig. 8, *bottom trace*, 3rd vertical line) midway through this last portion of the syllable coincides with the opening of the right side and the switch from unilateral to bilateral phonation.

In the second syllable there is a more pronounced effect of switching between the two sides of the syrinx. In this

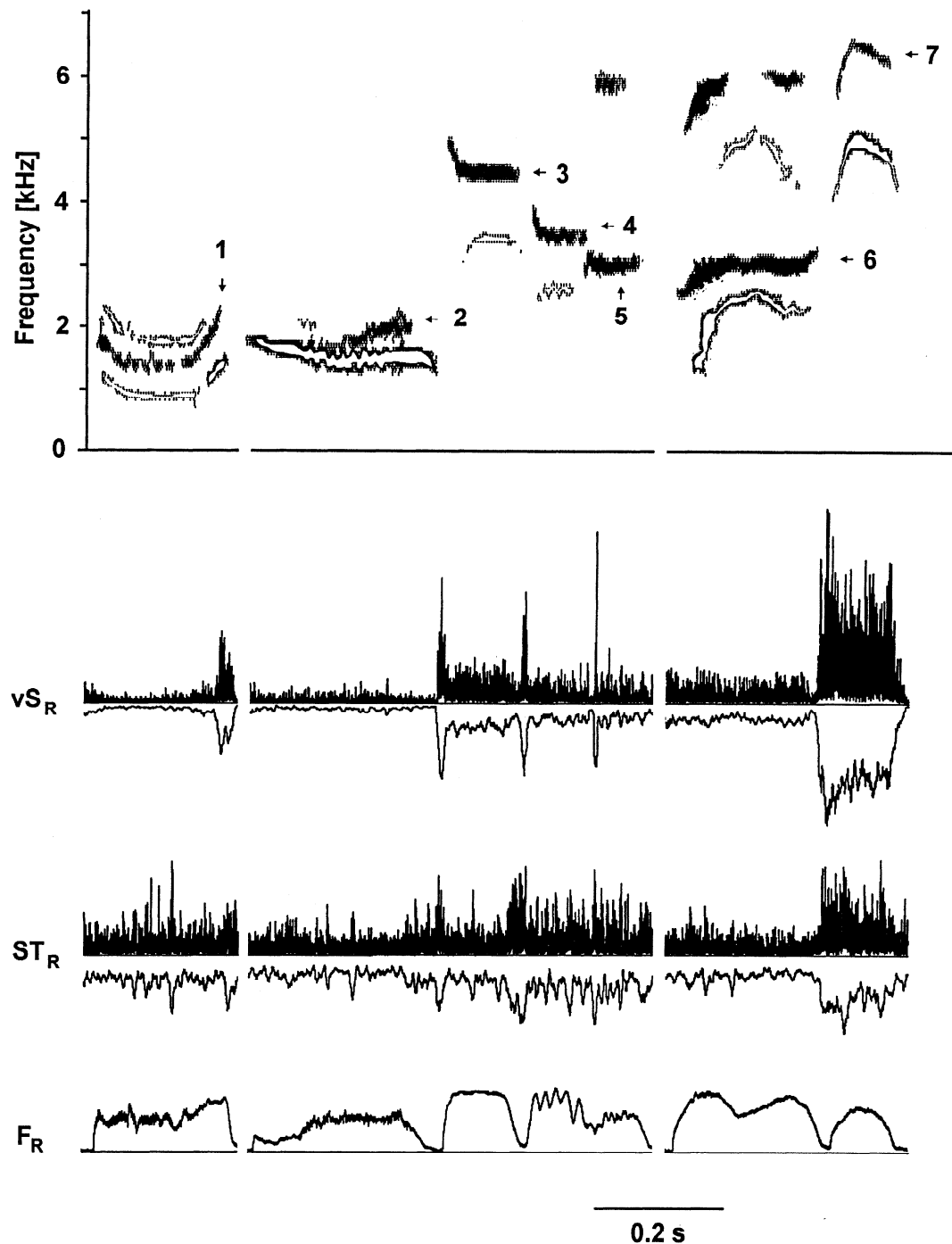


FIG. 4. Close correlation between f_1 and vS EMG activity is also present on the right side but is not pronounced in ST. Sound spectrograms with right airflow and EMG recordings in vS and ST are shown. The contributions of the left side are outlined on the spectrogram. Abbreviations as in Figs. 1 and 2.

case phonation switches from left to right and back to the left as indicated by the airflow. During each switch, amplitude drops to a minimum. Control of the rate of airflow by syringeal muscles must also be accompanied by changes of contraction of the pressure-generating abdominal expiratory muscles, because modulation of air sac pressure does not merely reflect changing syringeal resistance. Alternation of airflow between the sides usually does not generate very complicated AM patterns.

More complex patterns of AM are produced by repeatedly

varying syringeal resistance during the course of phonation. This results in oscillations in the rate of airflow, which may fluctuate only a little or may decline to almost zero during each cycle. These flow oscillations generate corresponding modulations of sound intensity but the amplitude of intensity modulation is not correlated with the amplitude of flow oscillation ($R^2 < 0.05$ in 3 thrashers), probably because of complex interactions of the different syringeal muscles and acoustic interactions with the contralaterally generated sound.

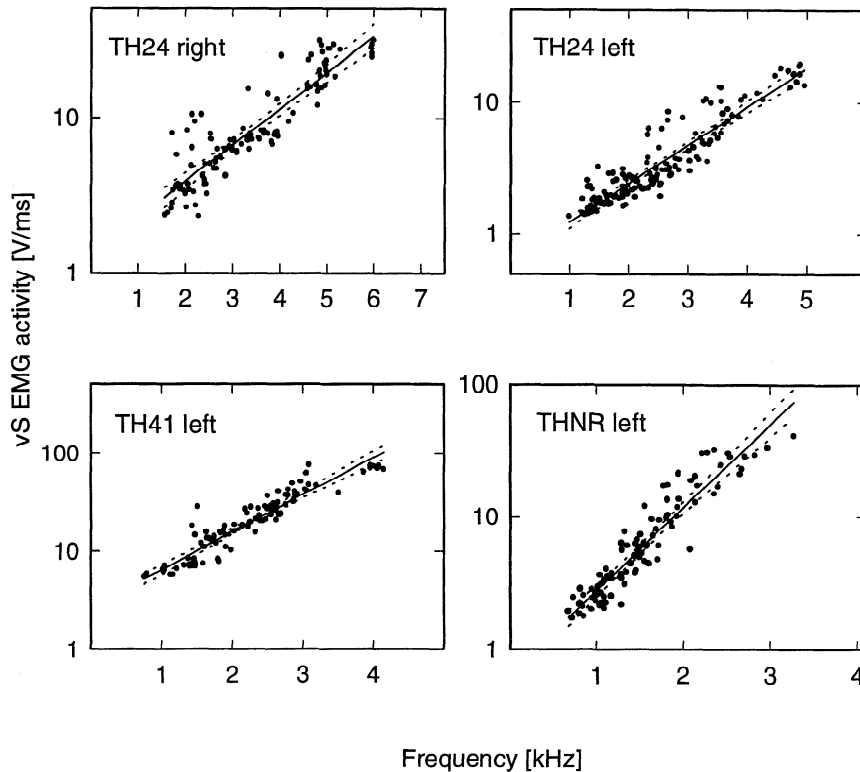


FIG. 5. EMG amplitude in vS is exponentially (logarithmic scale on vertical axis) correlated with the f_1 of the ipsilaterally generated sound. Four examples of different thrashers are shown. EMG activity was averaged over segments of relatively constant frequency and normalized for different durations. Best fit exponential correlations through the data points are shown (—) along with 99% confidence intervals (---).

The fluctuations in airflow appear to be primarily generated by activity of the syringeal muscles that regulate syringeal aperture and gate sound production by controlling airflow (Goller and Suthers 1996; Suthers et al. 1994). These muscles include the main adductors, dS and dTB, which are activated in short bursts in synchrony with decreasing airflow (Fig. 9a). Activity in the abductor muscle, vTB, is more variable in syllables with flow oscillation and may modify syringeal resistance as set by the dorsal adductor muscles (Fig. 9; Goller and Suthers 1996). EMG bursts in dorsal muscles coincide with the onset of the decrease in both the rate of airflow and in sound intensity (Fig. 9, *a* and *c*).

Although low-amplitude flow oscillations can occur when there is only weak adductor muscle activity (dS, dTB), as indicated by low-amplitude EMG bursts (not shown), frequently such AM syllables are accompanied by a complex

pattern of simultaneously strong electrical activity in several syringeal muscles including the adductors (Fig. 9b). There are many intermediate examples of varying strength of EMG activity in different syringeal muscles. The two syllables in Fig. 9b, for example, are produced with varying amplitude of bursts in dorsal muscles and high-amplitude bursts in vTB.

When all syringeal muscles are strongly activated, flow oscillations are of very small amplitude or absent. At such times high-amplitude bursts in adductor (dS and dTB) and abductor (vTB) muscles typically occur simultaneously (Fig. 9, *b* and *d*). This synchronized activity of antagonists presumably reduces the adductive effect of dorsal muscles. Thus oscillations in the rate of airflow are much lower than predicted from the amplitude of EMG bursts in the dorsal muscles (Goller and Suthers 1996). The frequency of AM is usually higher and the modulation amplitude is generally lower than in syllables with pronounced flow oscillations (compare Fig. 9, *a* and *b*). EMG bursts are aligned close to the minimum in sound amplitude, suggesting a different syringeal mechanism of AM generation than described above (Fig. 9d). Not all changes in sound intensity in syllables *a* and *b* (Fig. 9) are accompanied by EMG bursts, suggesting that some AM is controlled by other muscles or a different mechanism.

AM DUE TO ACOUSTIC INTERACTION. AM can also result from acoustic interactions of two sounds produced independently on each side of the syrinx and therefore not be a direct effect of muscle action. Difference tone phenomena and probably other acoustic interactions of sounds in the two syringeal halves contribute in a complex way to the AM structure of the syllable shown in Fig. 10. Neither the EMG

TABLE 1. Correlation of syringeal muscle activity with fundamental frequency of ipsilateral sound

Syringeal Muscle	R^2 Average	Range	Slope	Number of Data Sets
vS	0.84	0.71–0.95	Positive	7
vTB	0.53	0.39–0.70	Positive	4
dS, dTB	0.26	0.16–0.41	Positive	4
TL	0.60	0.59, 0.62	Positive	2
ST	0.30	0.00, 0.60	Positive	2

Syringeal muscle activity presented on a logarithmic scale. vS, syringealis ventralis; dS, m. syringealis dorsalis; vTB, m. tracheobronchialis ventralis; dTB, m. tracheobronchialis dorsalis; TL, m. tracheolateralis; ST, m. sterno-trachealis. Data sets include recordings from different individuals and left and right muscles from 1 individual (vS, vTB).

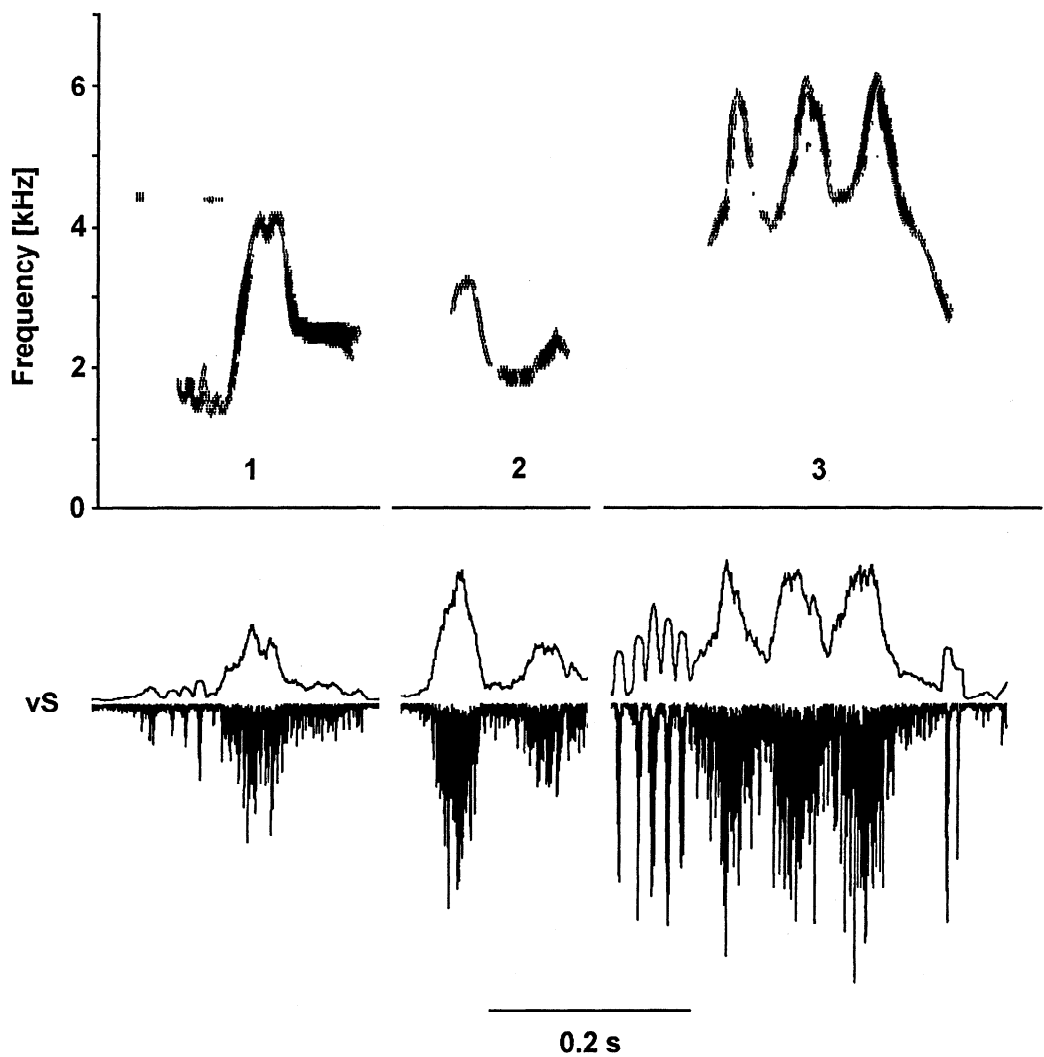


FIG. 6. EMG activity in vS can be used to predict the frequency modulation pattern of the ipsilaterally generated sound. Three examples generated on the left (numbers 1 and 2) and right (number 3) sides of the syrinx and from different thrashers are shown with rectified (downward deflection) and integrated (upward deflection) representation of the electrical activity in vS.

activity of the three monitored muscles nor changes in the rate of airflow correspond to the AM of this vocalization. Sound production is initiated on the left side and, as the

TABLE 2. Correlation of syringeal resistance with fundamental frequency of ipsilateral sound

Bird	Side	R^2	Slope	Number of Syllables
TH22	Left	0.02	Positive	58
	Right	0.14	Positive	85
TH50	Left	0.17	Positive	89
TH24	Left	0.26	Positive	158
	Right	0.06	Positive	108
TH41	Left	0.14	Positive	154
THNR	Left	0.52	Positive	100
	Right	0.43	Positive	124

Syringeal resistance was approximated as $R = P/F$, where P is air sac pressure and F is ipsilateral nonlinearized rate of airflow. Resistance values were log-transformed, which resulted in a linear relationship.

right side starts to generate sound, the second harmonic of the left note (f_{2L}) appears and is of greater intensity than the f_1 (f_{1L}) (Fig. 10a, shaded column). The note generated on the left side (Fig. 10b, spectrogram labeled S_L) is also registered by the right thermistor probe as soon as the right side is partially abducted (Fig. 10b, spectrogram labeled S_R). In addition, the right side generates its own independent note at higher frequency.

At the beginning of bilateral phonation (Fig. 10b, 1st 2 vertical lines), the rate of AM equals the frequency difference of f_{2L} and f_{1R} (difference tone). The AM associated with this beat note is reflected in the amplitude patterns of the left and right bronchial sound, but is more pronounced on the right side (Fig. 10b). As f_{2L} and f_{1R} become equal (between 2nd and 3rd vertical lines in Fig. 10b) there is a marked decline in sound amplitude, suggesting that the waveform of the left side and its cross talk signal on the right thermistor are out of phase by nearly 180°. This AM of the emitted sound appears to arise primarily in the right syrinx, as indicated by the amplitude envelopes of S_R , S_L ,

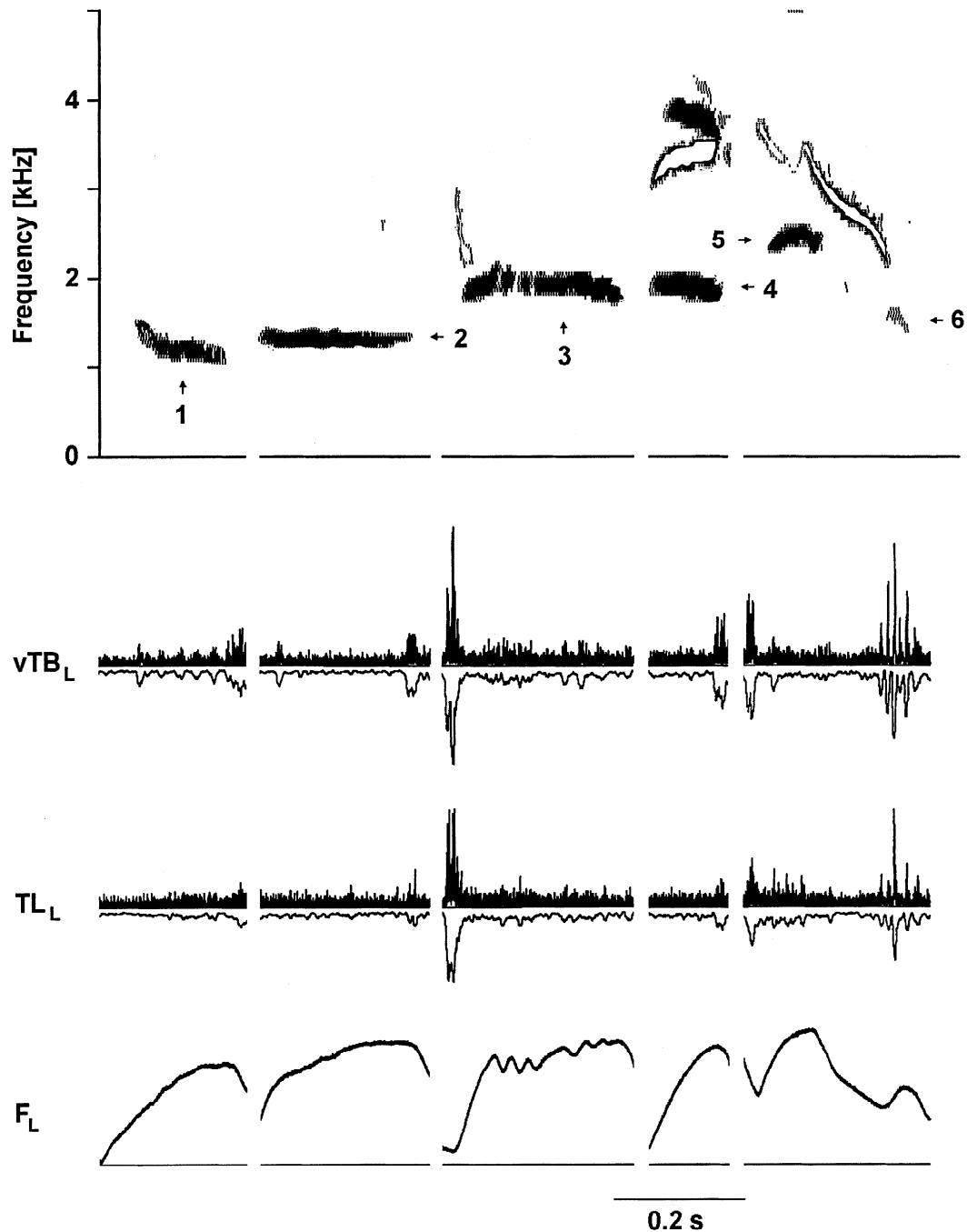


FIG. 7. EMG activity in vTB and TL does not closely reflect the f_1 of ipsilaterally generated sound. Syllables produced on the left side (right side contributions are outlined) are shown along with EMG recordings and left airflow patterns. Abbreviations as in Figs. 1 and 2.

and the vocalization (A) in Fig. 10*b*, *top*. If this interaction was due to destructive interference of phase-shifted left and right waveforms in the trachea we would not expect to see AM in the right bronchus similar to that in the vocalization. The fact that the AM pattern appears in the right bronchus suggests that at least a significant portion of the left/right acoustic interaction may occur in the bronchus by transmission of the left sound into the interclavicular air sac and through the thin wall of the right bronchus. It is not clear why transmission of sound between the bronchi occurs in some syllables from one side to the other but not in the

reverse direction and not at all in other syllables (see also Suthers et al. 1994).

DISCUSSION

Fundamental frequency

The close correlation of sound frequency and EMG strength in vS and the similarity between vS EMG patterns and FM provide strong evidence that vS activity controls frequency. Thus vS activity may regulate tension of the me-

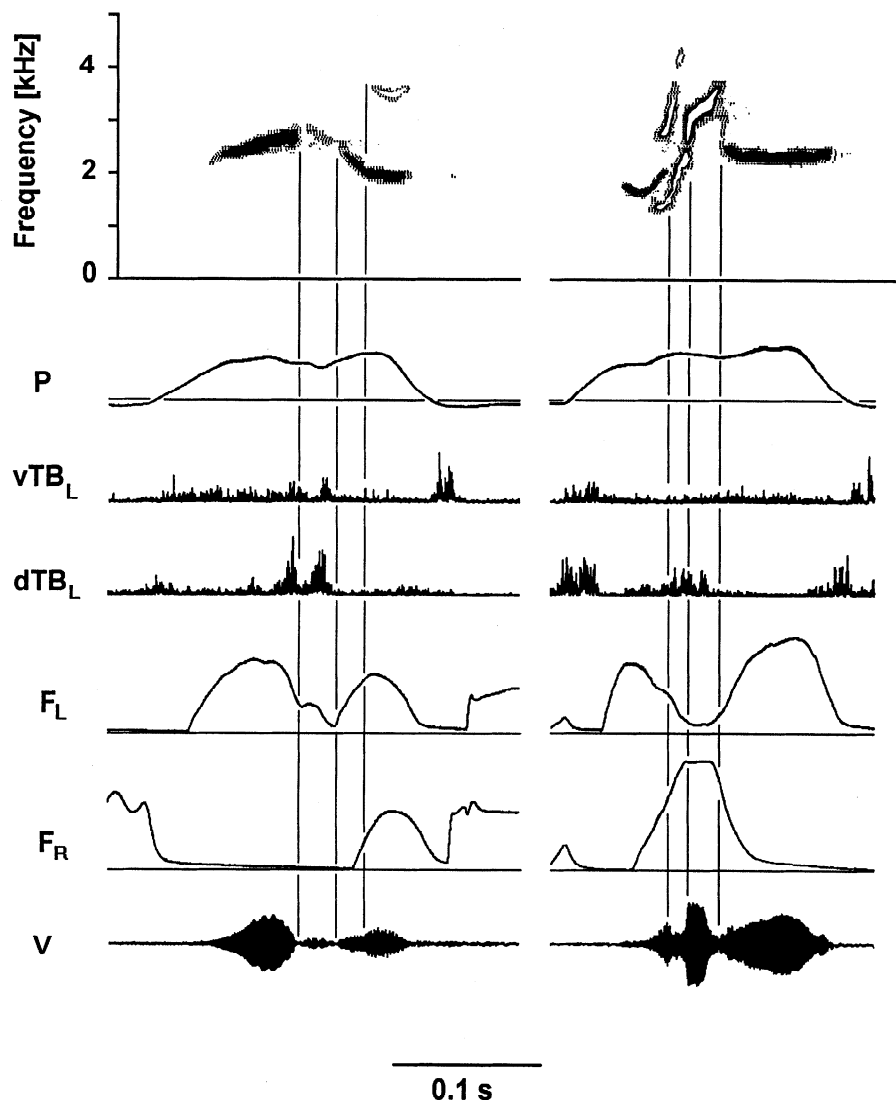


FIG. 8. Unilateral changes in the rate of airflow and switching of sound production between the 2 sides of the syrinx produce amplitude modulation (AM). Bilateral airflow recordings from 2 syllables illustrate these mechanisms. The EMG traces show adductor (dTB) and abductor (vTB) activity on the left side. Vertical lines: changes in sound amplitude. Abbreviations as in Figs. 1 and 2.

dial tympaniform membranes, which are thought to be the sound-generating structures in the passeriform syrinx (Brackenbury 1989; Greenewalt 1968; King 1989; Miskimen 1951). This is in agreement with the proposed role of vS derived from morphological studies of the crow syrinx (*Corvus brachyrhynchos*) (Chamberlain et al. 1968).

However, the production of high-frequency sounds is not only accompanied by increased activity in vS muscles. EMG amplitudes in all syringeal muscles show positive correlations with sound frequency (Table 1). Increased activity in these other muscles may be needed to stabilize the syringeal framework during strong contraction of vS, the largest of the syringeal muscles, when high frequencies are produced.

Correlation coefficients for EMG activity and frequency are lowest for dorsal muscles (average $R^2 = 0.26$, Table 1), which are the main adductors (Goller and Suthers 1996). There is also a slight trend for higher frequencies to be associated with higher syringeal resistance values (average $R^2 = 0.22$, Table 2). A significant portion of the covariance of dorsal EMG activity with frequency is eliminated by accounting for changes in syringeal resistance. This is consistent with the proposed biomechanical effect of contraction

of dorsal muscles, namely to regulate syringeal aperture by rotating the lateral labium into the bronchial lumen (Chamberlain et al. 1968), and suggests that dorsal muscle activity does not directly affect tension of the medial tympaniform membranes. Activity of dorsal muscles may be occasionally higher than expected, on the basis of resistance to airflow, because the antagonistic abductor muscles (vTB, TL) (Goller and Suthers 1996) are also more active during high-frequency vocalizations (Table 1).

Frequency modulation

The striking similarity between the vS activation patterns and the FM of the sound indicates that changes in frequency are actively controlled by this muscle. The only exception appears to be small 100- to 200-Hz FM that coincides with AM during rapidly oscillating airflow when there is no corresponding EMG pattern in vS. This FM may be caused passively by changing tension of the medial tympaniform membranes as a result of aerodynamic forces produced by fluctuating airflow and pressure across the membrane.

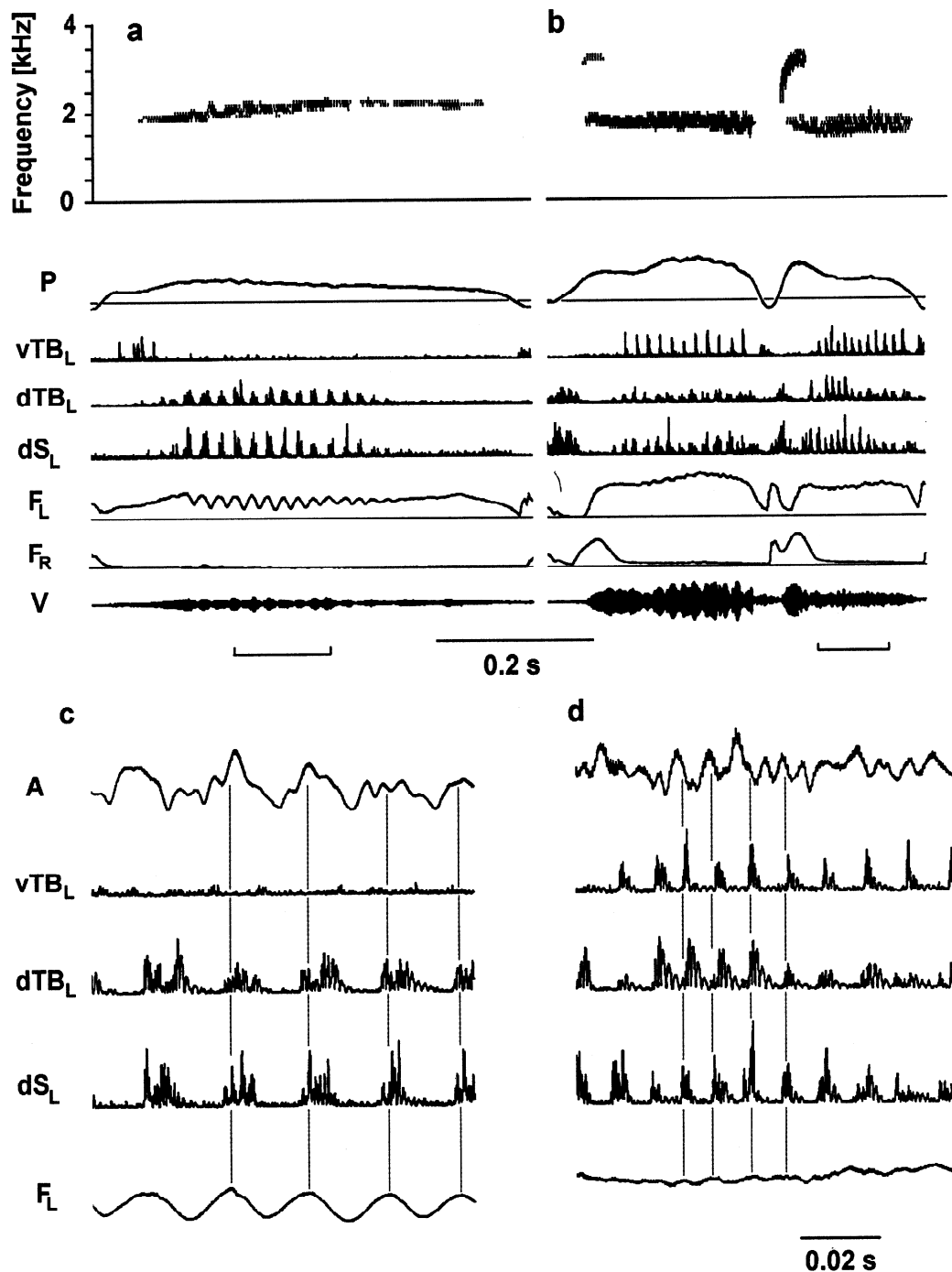


FIG. 9. AMs can be generated directly by activity of syringeal muscles. *a*: large oscillatory changes in the rate of airflow are accompanied by bursts of EMG activity in the adductors (dS and dTB) and generate AM. *b*: low-amplitude flow modulations and strong EMG bursts in adductors and abductor (vTB) can also generate AM. *c* and *d*: expanded view of segments of the 2 syllables (indicated by brackets in *a* and *b*) illustrating the different activation of syringeal muscles and the differences in synchronization of vTB and dorsal muscles, dTB and dS. A, rectified (time constant 0.1 ms) and integrated (time constant 1 ms) representation of the oscillogram; other abbreviations as in Figs. 1 and 2.

Comparison with zebra finch

In zebra finches, activity of syringeal muscles (Vicario 1991b) and their representation in the hypoglossal nucleus (Vicario and Nottebohm 1988) have been studied. Chronic EMG recordings of the two largest syringeal muscles (vS and dS) provide the only other study of muscle activity in spontaneously singing songbirds. The

analysis of Vicario (1991b) is mainly focused on temporal correlations between muscle EMG activity and sound generation. In zebra finches the highest-amplitude EMG bursts coincide with the onset and offset of sound production (see discussion in Goller and Suthers 1996). Illustrations of EMG activity and spectrograms (Figs. 5 and 7 in Vicario 1991b) indicate that vS activity in zebra finches may also be correlated with the f_1 of sound. How-

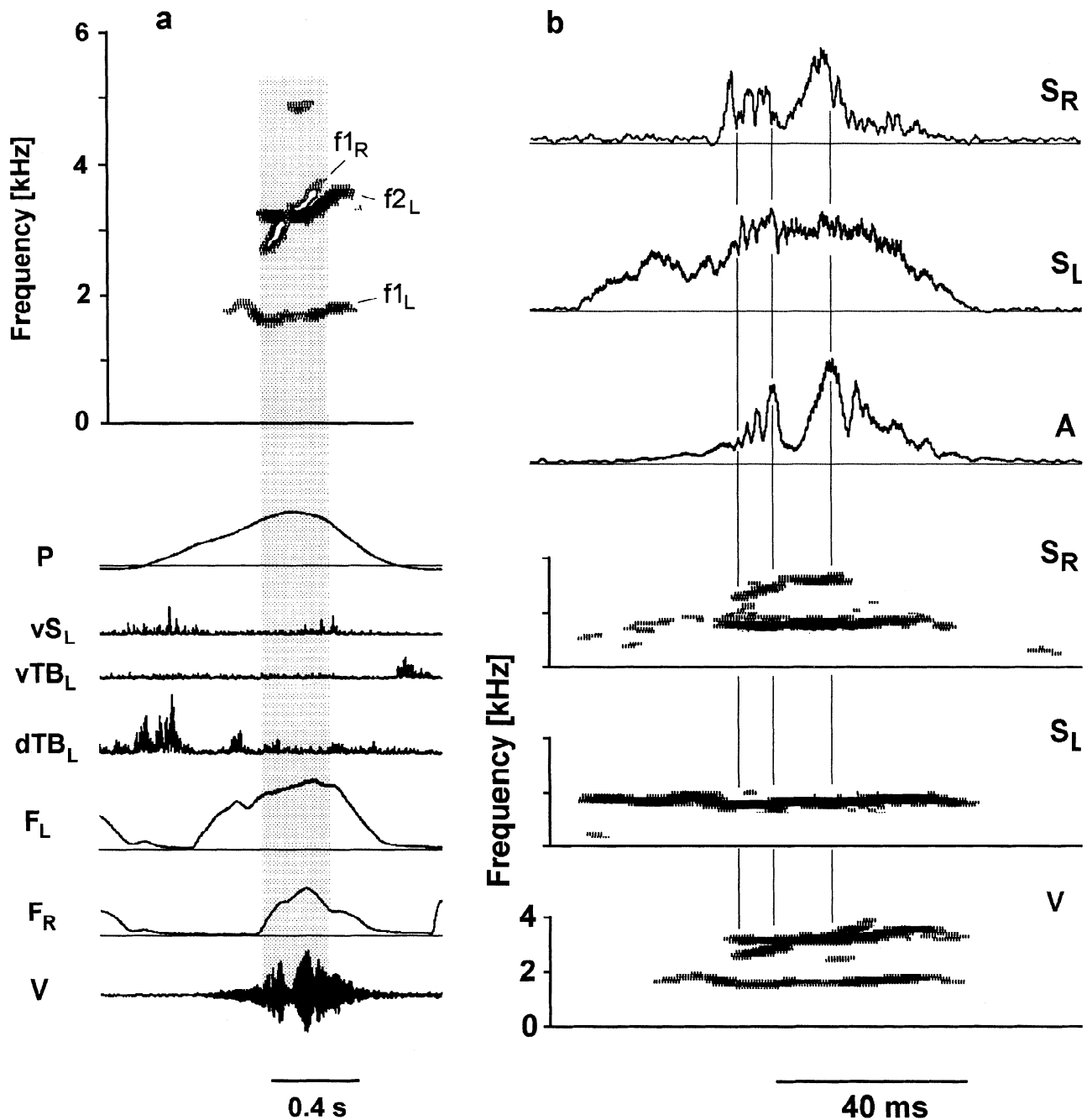


FIG. 10. AM can also be generated by acoustic interaction of the 2 sound-generating sides of the syrinx. *a*: example of complex acoustic interactions of the 2 simultaneous sounds. The period of right side contribution is shaded. *b*: expanded view of spectrograms together with rectified and integrated representations of the sound signals. A, amplitude of sound; S_L and S_R , left and right bronchial sound; other abbreviations as in Figs. 1 and 2. Periods of the beat note AM between the 1st 2 vertical lines are 1.94, 2.28, and 3.43 ms, corresponding to frequency differences of 515, 438, and 291 Hz, respectively. Slight delays between peaks in the emitted sound (Δ) and S_R are presumably due to propagation time differences. Amplitude differences between the cross talk signal from the left side and the sound generated on the right side on the S_R spectrogram are not accurate reflections of the respective amplitudes because the response of the thermistor declines nonlinearly with increasing sound frequency. The high-frequency note on the S_R spectrogram is a combination of the right-side-produced sound and the second harmonic of the cross talk signal from the left-side-generated sound. However, the initial portion of f_{2L} and the tail end of f_{1R} are cut off, presumably because of the limited sensitivity of the thermistor at lower intensities.

ever, in zebra finches the respective contributions of the left and right sides of the syrinx are not known, making detailed analysis of the correlation of EMG activity with sound frequency impossible.

Amplitude modulation

The generation of AM in brown thrashers is very complex because several different mechanisms can contribute simul-

taneously to the AM pattern of a single syllable. In syllables with oscillatory changes in airflow, muscles that control the aperture of the syringeal lumen actively generate AM by modulating the rate of airflow through the syrinx. In most of these syllables, complex interactions of all syringeal muscles lead to AM patterns that do not have a simple relationship to the airflow oscillation. Although modulation of airflow appears to produce AM, the amplitude of AM is not correlated with the amplitude of flow oscillation. This lack of correlation is consistent with the variability in EMG activation patterns that accompany different syllables. Because syllables with high-amplitude EMG bursts in gating and non-gating muscles are characterized by very low-amplitude flow oscillations, yet also by significant AM, antagonistic interactions between the syringeal muscles must take place. A variety of activation patterns constitutes a continuum of interaction from the simplest pattern, where adductor activity alone causes oscillating airflow, to very high-amplitude activation of all syringeal muscles.

The syringeal mechanics by which AM is generated probably also differ according to the temporal relationships of EMG bursts to sound AM. In syllables with marked airflow modulation, maxima in sound intensity are aligned with the peak rate of airflow. However, in syllables with weakly modulated airflow but high-amplitude EMG bursts in the syringeal muscles, there is no consistent alignment of changes in sound intensity and airflow. Slight movements of various syringeal structures caused by simultaneous activity of all syringeal muscles could modulate sound intensity. Thus, with the use of this mechanism, AM may sometimes be generated by direct muscle action without simultaneous changes of syringeal airflow.

In addition to this complex direct action of syringeal muscles in producing AM, acoustic interactions of the sounds generated on the two sides of the syrinx can further contribute to the amplitude pattern. AM resulting from difference tones is frequently produced by thrashers (Suthers et al. 1994) and has also been described for a number of other species (Greenewalt 1968; Stein 1968).

In some syllables, sounds generated on one side of the syrinx are also detected in the contralateral bronchus. It is possible this may occur if sound in the interclavicular air sac surrounding the bronchi and syrinx is transmitted through the thin flexible wall of the contralateral bronchus. The degree to which vocal intensity is modulated by such cross talk doubtless depends on the phase relationship between the syringeally generated sounds.

The amplitude structure of vocalizations containing multiple harmonics may also be under direct syringeal control. In brown thrashers energy maxima shift between harmonics in conjunction with opening and closing of the contralateral side (Fig. 10). Such shifts, which are frequent at low f_1 s, may reflect changing resonant properties of the upper vocal tract, probably as a consequence of increased aperture either at the syringeal and/or simultaneously at the glottal end of the trachea. In the song of the zebra finch (*Taeniopygia guttata*), many syllables have between 10 and 12 harmonics and the relative amplitude of these harmonics varies from syllable to syllable and between birds. This amplitude profile of formants is severely disrupted after tracheosyringeal nerve cuts, suggesting an active role of the syringeal muscles in

its generation (Williams et al. 1989). In oilbirds (*Steatornis caripensis*), amplitude spectra of vocalizations with complicated harmonic structure are probably generated by resonant properties of the vocal tract. Formant frequencies differ between individuals as a result of varying structural asymmetries (Suthers 1994).

Central control of phonetic structure

Our recordings directly confirm in intact birds that the syringeal muscles play an important role in determining the phonetic structure of sounds. This is consistent with alterations of learned vocalizations after unilateral or bilateral sectioning of the tracheosyringeal branch of the hypoglossal nerve, which innervates ipsilateral syringeal muscles. In brown thrashers (Suthers and Hartley 1990) and zebra finches (Simpson and Vicario 1990; Williams 1990), sounds generated on the denervated side are significantly lower in f_1 and lack FM.

Aside from this accurate coordination of the potentially complex activation patterns of all six syringeal muscles of one side, central song control also involves integration of the activity of the two sides of the syrinx. Song production in brown thrashers is lateralized in the sense that the two sides produce independent sounds and sound generation is switched from side to side (Goller and Suthers 1995; Suthers 1990; Suthers et al. 1994). Thus, not only must muscles on the phonating side of the syrinx be coordinated, but their excitation must also be integrated with the activity of contralateral muscles, because the phonetic structure of a syllable depends on precise gating of airflow through the two sides and precise control of both sound-generating membranes.

In addition to lateralized independent action of the two putative sound-generating membranes in the syrinx, we document here that the generation of AM can be lateralized through interaction of the two sides. Unmodulated sound, generated on one side, interacts on the contralateral side with the sound produced there, generating AM that determines the AM pattern of the emitted vocalization (Fig. 10).

Frequency and amplitude characteristics do not only depend on syringeal activity. Respiratory patterns have an important influence on sound intensity and the temporal structure of song (Brackenbury 1989; Simpson and Vicario 1990). Acoustic properties of the respiratory tract must also influence the amplitude and formant structure of the radiated sound. They may be determined passively by its morphology (Suthers 1994) or potentially by actively changing its dimensions (Hinsch 1972; Nowicki 1987) and/or coupling to the environment (Hausberger et al. 1991; Westneat et al. 1993).

Consequently, central song control involves not only the generation and coordination of syringeal muscle activity but also the integration and coordination of all motor systems that contribute to sound production.

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