

IN SITU BIOMECHANICS OF THE SYRINX AND SOUND GENERATION IN PIGEONS

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Summary

The *in situ* biomechanics of the vocal organ, the syrinx, was studied in anesthetized pigeons using fiberoptic instruments. The role of syringeal muscles was determined by electrical stimulation, and phonation was induced by injecting gas into the subsyringeal air sacs. This study presents the first direct observations of the biomechanical processes that occur in an intact syrinx.

Contraction of one of the syringeal muscles, the m. tracheolateralis (TL), withdraws the lateral tympaniform membranes (LTM) from the syringeal lumen, causing opening of the syringeal airways. Shortening of a second muscle, the sternotrachealis (ST), draws the syringeal cartilages closer to each other, causing the LTM to fold into the syringeal lumen. Maximal ST contraction does not lead to complete closure of the syrinx.

As air-sac pressure is increased by the injection of gas, the LTM are drawn into the syringeal lumen and balloon in a rostral direction until they touch, thus forming a fold-like valve. Air-induced phonation is always associated with vibrations of the membrane folds, suggesting that pulsatile

release of air into the trachea by vibratory motion of the LTM generates sound. During air-induced phonation, strong stimulation of the TL terminates sound generation by abducting the LTM, whereas weak stimulation changes the geometry of the membrane folds, which is accompanied by changes in the acoustic structure of the sound. Stimulation of the ST has little effect on air-induced sounds.

The LTM appear to be the main sound generators, since disabling the medial tympaniform membranes (MTM) with tissue adhesive does not prevent phonation or change the frequency and amplitude structure of display coos in spontaneously vocalizing pigeons. Moreover, the activity of the syringeal muscles appears to have a mainly modulatory function, suggesting that the basic sound-generating mechanism is similar in both air-induced and natural phonation.

Key words: bird, vocalization, vocal mechanism, endoscopic analysis, syringeal muscle, pigeon, *Columba livia*.

Introduction

In birds, sound communication is an important aspect of behavior and has been the subject of extensive study in several disciplines. Understanding the mechanisms of sound generation is often central to the interpretation of the experimental results from these investigations. The physical mechanisms of sound production have been studied indirectly by acoustic analysis (e.g. Greenewalt, 1968; Stein, 1968), by monitoring physiological correlates of phonation (e.g. Gaunt *et al.* 1973, 1976, 1982; Lockner and Youngren, 1976; Brackenbury, 1978, 1980; Suthers and Hector, 1982, 1985; Suthers, 1990; Goller and Suthers, 1995, 1996), by investigating dissected specimens (Beebe, 1925; Ruppell, 1933; Miskimen, 1951; Gross, 1964; Abs, 1980) or syrinx models (Dürrwang, 1974; Abs, 1980) and by theoretical analysis (e.g. Brackenbury, 1979; Casey and Gaunt, 1985; Fletcher, 1988). These studies have produced a number of hypotheses about the biomechanical events leading to sound

generation and the associated motor control which now await testing by means of more direct studies of the vocal organ of birds, the syrinx.

Two main physical mechanisms of sound generation have been proposed. (1) In the mechanical models, sound is generated by air-driven membrane vibrations (Greenewalt, 1968; Stein, 1968; Gaunt and Gaunt, 1985; Fletcher, 1989). Sound is generally thought to be generated by alternating compression and rarefaction of air as the membranes move into and out of the syringeal lumen ('classical model' of avian phonation, e.g. Greenewalt, 1968; Stein, 1968). Alternatively, a pulsatile release of air into the suprasyringeal airways through a valve formed by the syringeal membranes ('pulse tone' model, e.g. Grütznér, 1879, cited in Paulsen, 1967; Beebe, 1925; Klatt and Stefanski, 1974; Gaunt and Gaunt, 1985; Gaunt, 1987) has been proposed. The distinction between these two mechanisms of sound production may be somewhat artificial,

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since they may be the extreme ends of a continuum of intermediate vibratory modes of the syringeal membranes (Gaunt and Gaunt, 1985; Gaunt, 1987). (2) In the 'aerodynamic hypothesis', constrictions in the syringeal airway combine with high-velocity airflow to generate vortices in a manner similar to sound generation by a hole-tone whistle (Nottebohm, 1976; Gaunt *et al.* 1982). Considering the variety of sounds in the vocal repertoire, even within one species, different mechanisms may be responsible for the generation of different sounds (Klatt and Stefanski, 1974; Gaunt and Gaunt, 1977).

The vocal behavior of pigeons and doves (family Columbidae) is of interest in the context of sound-generating mechanisms because the morphology of their syrinx is relatively simple and well studied but, nevertheless, the physical mechanisms of phonation are debated (Wunderlich, 1884; Warner, 1972; Abs, 1980; Gaunt *et al.* 1982; Baptista and Abs, 1983; Ballintijn *et al.* 1995). The vocal organ of pigeons is located at the bronchotracheal junction and consists of two modified tracheal cartilage rings and several bronchial semi-rings. The two syringeal muscles, the m. tracheolateralis (TL) and m. sternotrachealis (ST), are extrinsic muscles and are believed to regulate the syringeal aperture. Contraction of TL is assumed to abduct the lateral tympaniform membranes (LTM), whereas ST shortening draws the trachea caudad causing adduction of the LTM (Gaunt *et al.* 1982; Warner, 1972).

The medial tympaniform membranes (MTM) are situated on the medial side of each bronchus and are thin elastic membranes. The LTM, however, are thickened and, on the basis of this evidence, the MTM were identified as the presumed sound generators in pigeons and doves (*Streptopelia decaocto*) (Warner, 1972; Ballintijn *et al.* 1995). Vibrations of the MTM are thought to be induced following the 'classical avian model'. In contrast, a whistle-like sound-generating mechanism was proposed in an attempt to explain the pure-tone vocalizations of doves (*Streptopelia risoria*). The bronchotracheal junction is seen as a potential constriction for aerodynamic sound generation, whereas the LTM presumably play a role in regulating sound amplitude by acting as a muffling valve (Gaunt *et al.* 1982).

To test these conflicting hypotheses, it will be necessary to study the sound-generating structures directly in the intact syrinx. Direct imaging of sound-generating structures has been applied successfully to the study of mammalian sound production, in particular human speech and singing. The first attempts to visualize the human larynx date back to the last century (for a historical account, see Proctor, 1980) and, more recently, the vocal folds have been imaged using high-speed cinematography and other techniques allowing detailed analysis of their vibratory behavior during the generation of a variety of sounds (e.g. Farnsworth, 1940; Hirose *et al.* 1991; Fukuda *et al.* 1991; Hirano *et al.* 1991). Similar approaches in birds have been difficult, since, unlike the human larynx, the syrinx is located deep inside the body cavity near the heart, and access to the intact syrinx could not be gained using laryngoscopic methods (Paulsen, 1967).

Here we report the results of a first attempt to study the biomechanics of the intact syrinx in pigeons using endoscopic techniques. The syrinx was filmed using fiberoptic systems to investigate syringeal biomechanics *in situ*, and sound generation was studied by inducing phonation in anesthetized pigeons. The results directly demonstrate the biomechanical effects of the syringeal muscles and provide some insight into the sound-generating mechanism. In addition, we show that endoscopic investigation is a useful tool for the study of the vocal organ of birds.

Some of these results have been presented as a poster (Larsen and Goller, 1996).

Materials and methods

For endoscopic experiments, 12 male domestic pigeons (*Columba livia* Gmelin) were acquired from a local vendor in Odense, Denmark. The experiments were approved by the Danish Animal Experimentation Committee. Pigeons were anesthetized by intramuscular injections of a Rompun-Ketamine mixture (initial dose 20 mg kg⁻¹ xylazine hydrochloride and 40 mg kg⁻¹ ketamine hydrochloride; supplementary doses were given as needed to keep the animal deeply anesthetized). At the end of the experiment, the birds were killed and the syrinx of several specimens was dissected to allow syringeal morphology to be studied. Experiments to examine the role of the MTM were performed at Indiana University on three additional pigeons (White Carneaux, Palmetto) and followed federal regulations for animal experimentation.

Measurements of air-sac pressure and airflow

Both posterior thoracic air sacs were cannulated by inserting a flexible cannula made of silastic tubing (Dow Corning, type 508-005). One cannula was attached to a piezoresistive pressure transducer (Fujikura, type FPM-02PG). The other cannula was used to inject gas into the air sacs and was plugged when not in use.

In some animals, the rostral end of the trachea was exposed by an incision in the skin 1–2 cm below the glottis. A small opening was cut into the ventral side of the trachea by partially removing a tracheal cartilage. Through this hole, a microbead thermistor (Thermometrics, type BB05JA202, 0.125 mm diameter) mounted in the center of a piece of stainless-steel tubing was inserted for measurements of airflow. The thermistor was heated to a constant temperature by a feedback circuit (Hector Engineering). The current needed to maintain the temperature of the thermistor was proportional to the rate of airflow. The direction of airflow, inspiratory *versus* expiratory, could be determined from simultaneous measurements of air-sac pressure.

Muscle electromyography and stimulation

The caudal end of the crop was dissected free and pushed to the side, and the interclavicular air sac was opened to expose the rostral ends of the syringeal muscles. Into both pairs of

syringeal muscles, the m. tracheolateralis and m. sternotrachealis, bipolar wire electrodes (Teflon-coated silver wire, 0.075 mm diameter) were inserted near their rostral insertion sites and fastened to the outermost fascia using cyanoacrylate tissue adhesive (Nexaband, Veterinary Products Laboratory). The wires were routed out of the air sac, which was then closed using tissue adhesive.

During quiet respiration, electrical signals from both syringeal muscles (EMGs) were recorded unilaterally after differentially amplifying (custom-built amplifier producing 1000× amplification) the electrode output. Syringeal muscles were stimulated during quiet respiration and air-induced phonation. Electrical stimuli were delivered bilaterally using a stimulator unit (DISA, type 14E11). The amplitude of stimulus pulses was between 0.5 and 1.5 mA, the pulse duration was 0.5 ms and pulse trains were delivered at frequencies between 10 and 100 Hz with train durations ranging from 0.5 to 1 s.

Endoscopic filming of the syrinx

For endoscopic recordings, pigeons were positioned on their back. The outside of the syrinx was filmed by inserting an arthroscope (Olympus, type A7595A, 4 mm diameter, 30° viewing angle) through the incision in the interclavicular air sac. The arthroscope was connected to an image control unit (Olympus, type OTV-S4), a light source (Olympus, type CLV-S) and a monitor (Olympus, type OEV-141). In a pilot study, internal tracheal views of the syrinx were initially recorded using a bronchoscope (Pentax, 3.4 mm outer diameter). The bronchoscope was inserted into the opening of the trachea and guided close to the syringeal area. After the feasibility of this approach had been determined using the bronchoscope, internal studies were made with an angiofiberscope (Olympus, type AF 14, 1.4 mm outer diameter, 75° field of view) connected to an image control unit (Olympus, OTV-A), a 300 W xenon light source (Olympus, type CLV-A) and a monitor (Olympus, type OEV-141).

Air-induced sound-generation

Phonation was induced by injecting oxygen into the subsyringeal air sacs. The rate of airflow was adjusted to the minimum necessary to trigger sound production (maximal flow rate 1 l min⁻¹). The flow rate of injected air needed to induce vibrations differed slightly between individuals, presumably as a result of minor leaks around the insertion sites of the cannulae and at the incision in the interclavicular air sac and to varying degrees of inflation of the crop. Insertion of the angioscope did not affect airflow noticeably (the dimensions of the dorsoventrally flattened trachea near the syrinx are 0.75–0.9 cm by 0.4–0.5 cm). The sound was recorded using a precision sound level meter (Brüel & Kjær, type 2235), the microphone of which was positioned between 3 and 5 cm in front of the beak.

Data recording and analysis

Air-sac pressure, EMGs, the rate of tracheal airflow and the induced sound were recorded either on an analog (Racal Store,

type 7D) or a digital (TEAC, type RD-135T) tape recorder. During quiet respiration, the microphone channel was used for comments. For data analysis, recordings were digitized (Data Translation, type DT-2821-G) using a sampling rate of 20 kHz and evaluated with SIGNAL v.2.2 software (Engineering Design).

Visual images were recorded using a Panasonic video recorder (type AG-6200, NTSC, 30 frames s⁻¹). The sound channel received input from the microphone or EMG recordings and was used to synchronize the video images with simultaneous recordings of the other parameters. Video segments were digitized (Vincent PCI board and Media100 software, Data Translation Inc.) on a Power Macintosh 9500 computer, and images were transferred into the software packages Photoshop v.3.0 and Corel v.5.0 for the preparation of figures.

Covering of the MTM with tissue adhesive

In three additional pigeons, the MTM were covered with tissue adhesive to prevent vibration. The syringeal area was accessed through an opening in the interclavicular air sac, but the MTM are situated so deep inside the body cavity that they could not be fully observed during the surgery. Tissue adhesive was applied through a long tube into the space between the bronchi. The interclavicular air sac was then closed using surgical sutures and tissue adhesive. Spontaneous vocalizations were recorded using a Realistic omnidirectional microphone onto a tape recorder (Marantz, type PMD-101) before and after the surgery and compared spectrographically using SIGNAL software. The animals were killed with an overdose of anesthetic after 2–3 days to determine how much of the MTM had been covered by tissue adhesive.

Results

Syringeal morphology

The syrinx of pigeons has been described previously in detail (Wunderlich, 1884; Warner, 1972; Abs, 1980). Morphological characteristics pertaining to the present study were confirmed by inspection of our dissected specimens. The syrinx is composed of the two most caudal rings of the trachea (T1 and T2) and the first few semi-rings of the bronchi (Fig. 1). T1 and T2 are enlarged and thickened ventrally and are not fully closed on the dorsal side, but separated by connective tissue. The lateral tympaniform membranes (LTM; also called external tympaniform membranes, ETM) span the lateral and dorsal area between T1 and T2. The LTM are relatively thick (up to 0.1 mm) masses of connective tissue and protrude into the lumen of the trachea. The m. tracheolateralis (TL) runs down the lateral side of the trachea and inserts on the LTM midway between T1 and T2. The insertion site is somewhat dorsal to the lateral midline of the membrane. The medial tympaniform membranes (MTM; also called internal tympaniform membranes, ITM) are situated on the medial sides of the rostral end of each bronchus, spanning the length

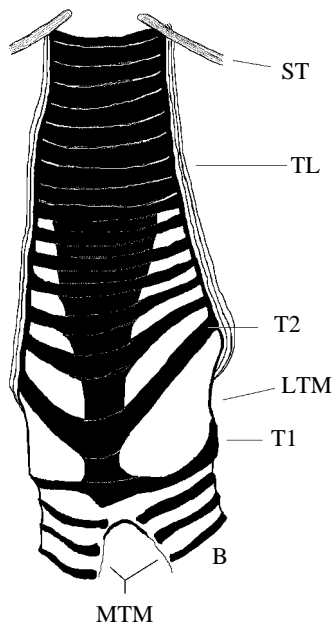


Fig. 1. Schematic ventral view of the pigeon syrinx illustrating the syringeal muscles and syringeal membranes. ST, m. sternotrachealis; TL, m. tracheolateralis; T1 and T2, first and second tracheal rings; LTM, lateral tympaniform membrane; MTM, medial tympaniform membranes; B, bronchus.

of approximately 6–8 bronchial semi-rings. The MTM are approximately 0.01 mm thick.

Quiet respiration

Electrical activity in the TL is restricted to the expiratory phase of the breathing cycle (Fig. 2). Each burst in TL is accompanied by movement of the LTM. The LTM is slightly withdrawn from the tracheal lumen and flicks back after the cessation of TL activity (Fig. 3). This abduction of the LTM generally coincides with an increase in positive subsyringeal air-sac pressure, usually at the end of the expiratory phase (Fig. 2). There is no measurable activity in ST during normal breathing under anesthesia (Fig. 2).

Stimulation of the syringeal muscles

Full contraction of TL was elicited by strong electrical stimulation, illustrating the full range of its biomechanical effect. As seen in a lateral outside view, during TL contraction, the second tracheal ring is moved rostrad and the LTM is stretched (Fig. 4). The internal view of the syrinx during strong electrical stimulation of TL shows the membrane position of maximal withdrawal (Fig. 5). This fully abducted state results in a larger diameter of the syringeal lumen than is achieved by spontaneous TL activity during quiet respiration (Fig. 3). The distance between the bronchial septum and the maximally abducted LTM measures 70 pixels in Fig. 5, which is significantly more than the 54 pixels in Fig. 3 despite the fact that the latter sequence was filmed with the angioscope lens positioned slightly closer to the syrinx.

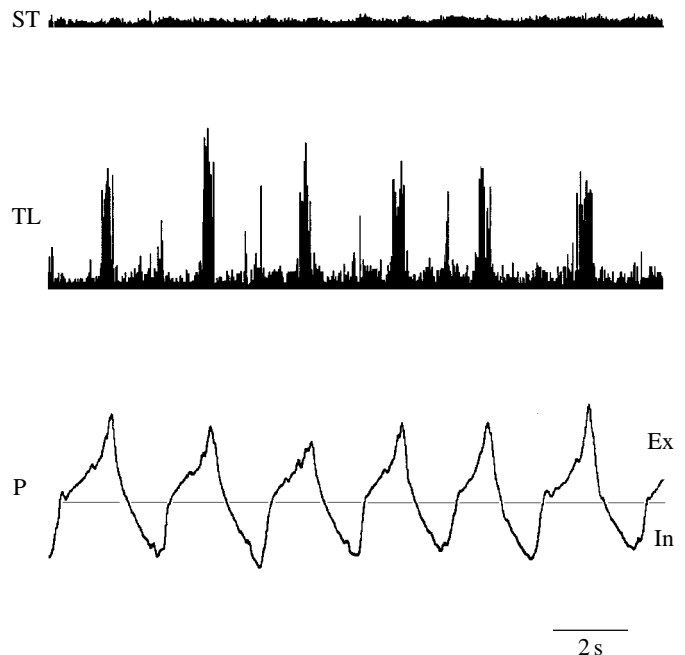


Fig. 2. During quiet respiration, m. tracheolateralis (TL) is active during the expiratory phase while m. sternotrachealis (ST) is not active. Subsyringeal air-sac pressure (P) is shown with electromyograms from the right syringeal muscles (rectified). The horizontal line on the pressure trace indicates zero difference from atmospheric pressure; values above the line are expiratory pressures (Ex) and those below indicate inspiration (In).

Stimulation trains were delivered with pulse frequencies varying between 10 and 100 Hz. Abductive movements of the membranes follow individual pulses up to approximately 40 Hz. At higher stimulation frequencies, the withdrawal of the membrane appears as one event for the duration of the stimulus train, indicating tetanic fusion of muscle contractions. Exact quantitative analysis of the fusion frequency is not possible given the low temporal resolution of the video equipment.

Contraction of ST induced by electrical stimulation draws the trachea caudad and also moves the tracheal rings of the syringeal area closer together (Fig. 4c,d). As T1 and T2 approach each other, the LTM fold and protrude farther into the lumen of the trachea (Fig. 6), but never fully close the tracheal lumen under our experimental conditions even when strong electrical stimulation produces maximal ST contraction (Fig. 6b).

Air-induced phonation

During air-induced phonation, the syrinx could only be filmed internally. During recordings of external views of the syrinx, sound induction was not successful because the insertion of the arthroscope into the interclavicular air sac caused air leaks and prevented phonation. The pressures necessary to induce phonation were 32- to 50-fold higher than the positive expiratory pressures measured during quiet respiration in the anesthetized birds.

Injection of gas into the posterior thoracic air sac and the

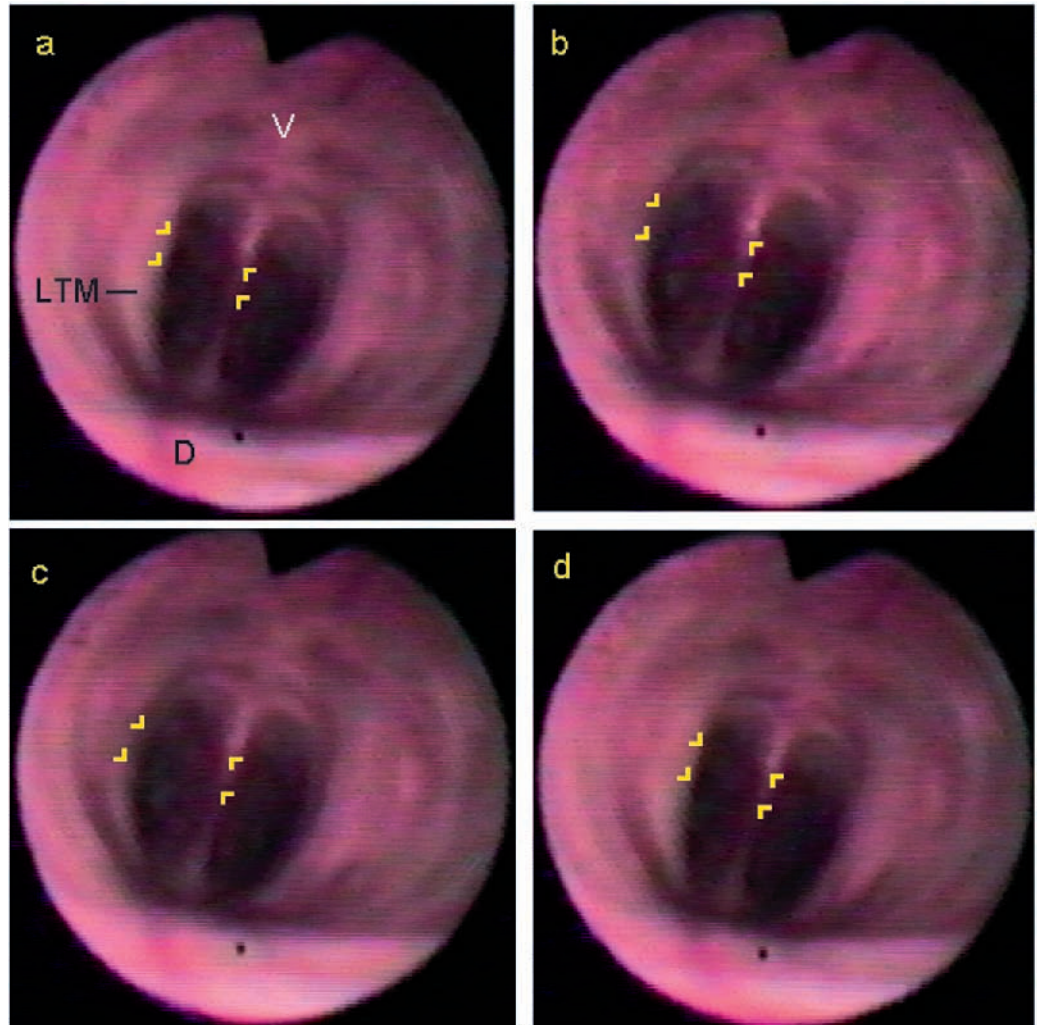


Fig. 3. The internal view of the syringeal area during quiet respiration illustrates the slight abductive event coinciding with *m. tracheolateralis* (TL) activity during expiration. During inspiration (a) the lateral tympaniform membranes (LTM) fold into the lumen. The LTM are slightly withdrawn during each expiration (b,c), and as TL activity stops (see Fig. 2) at the onset of inspiration they move back into the lumen (d). Yellow arrowheads mark the edge of the LTM and the bronchial septum to indicate the abductive action during expiration. The black dot indicates three broken fibers in the angioscope. The black notch is an indicator in the angioscope. D, dorsal; V, ventral. The range of syringeal dimensions is given in Materials and methods.

subsequent rise in subsyringeal pressure result in a repositioning of the LTM so that they bulge into the lumen of the syrinx and simultaneously move rostrad, closing the lumen to a narrow slot (Fig. 7). In addition, the dorsal wall of the syrinx also protrudes into the lumen under the increased pressure. Once the initial repositioning of the LTM has been achieved, vibratory movements of the LTM can be detected (Figs 8, 9). Under these conditions, audible sound is generated only when simultaneous vibrations of the LTM are present. Although the vibrations cannot be fully resolved with the low frequency resolution of the video equipment, some observations of the vibratory behavior of the membranes are possible. Vibratory movements are complex and have vector components both parallel and normal to the direction of air flow. The normal components generate small-amplitude oscillatory opening and closing of the airways. In some other instances, particularly as the injected airflow is reduced, large-amplitude vibrations of low frequency (30–80 Hz, as confirmed by sound recordings) normal to the direction of airflow can be observed shortly before vibrations stop altogether.

The complex vibratory behavior of the membranes agrees with the complex acoustic structure of the air-induced sound

(Fig. 10; see also below). If the angioscope was moved in a caudad direction during air-induced phonation, sound production stopped abruptly as the slot formed by the protruding edges of the LTM was penetrated by the fiberscope, stopping vibratory movements of the LTM. The fundamental frequency of the air-induced sound is between 200 and 400 Hz, and the harmonic structure is complex and quite variable (Fig. 10). Typically, there are multiple prominent higher harmonics present, and occasionally most of the energy is concentrated in the first two harmonics. Sounds produced in this manner are usually not pure tones and include low-intensity broad-band noise.

Strong stimulation of TL during air-induced phonation withdraws the LTM and terminates both vibrations and sound production (Fig. 8c). Weaker stimulation causes a slight abduction of the LTM, usually in the dorsal area. This slight withdrawal from the center of the lumen is also confirmed by an increase in the rate of airflow through the syrinx (Fig. 10). In addition to the slight dorsal parting of the LTM, they are also drawn slightly caudad, which is illustrated by the change in light reflection before and after the stimulation (Fig. 9). This effect can be seen more clearly in the film segments than in

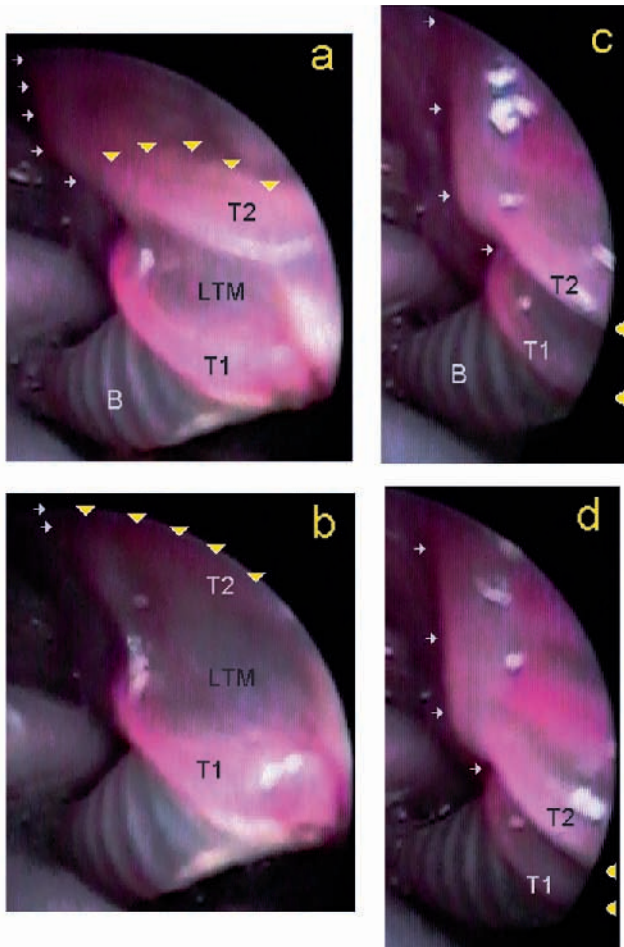


Fig. 4. Partial ventro-lateral view through the arthroscope of the outside of the syringeal area before (a,c) and after maximal electrical stimulation of *m. tracheolateralis* (TL) (b) and *m. sternotrachealis* (ST) (d). Yellow arrowheads mark tracheal ring T2 (a) and its rostral movement after TL stimulation (b). Yellow semicircles mark the absolute position of tracheal rings T1 and T2 and the distance between them before (c) and after (d) ST stimulation. White arrows show the lateral edge of the TL muscle and its insertion onto the lateral tympaniform membrane (LTM). The black circular margin on the right of each frame is the edge of the field of view. Rostral is upwards and medial is to the right in all frames. Other abbreviations as in Fig. 1.

individual still pictures. TL stimulation always affects the sound generated by either changing the harmonic structure or lowering the dominant frequency of the sound (Fig. 10). Weak stimulation with pulse repetition frequencies below 40 Hz resulted in a 100% amplitude modulation of the sound, whereas sound generation stopped at higher stimulation frequencies for the duration of the stimulus train correlated with the withdrawal of the LTM.

Stimulation of ST alone causes no detectable change in the sound generated under our conditions. This is true in all three individuals where ST stimulation was performed. The effectiveness of the stimulus in eliciting muscle contractions was confirmed before and after air-induced phonation.

Elimination of MTM as a vibratory source

The endoscopic analysis suggests that the LTM are a sound source during air-induced phonation. The role of the MTM during this phonation could not be determined since our approach through the trachea did not enable us to see the MTM, and advancing the angioscope past the LTM terminated sound generation. The role of the MTM was therefore investigated by preventing vibrations of the MTM in spontaneously calling individuals.

In two pigeons, between 50 and 75% of the MTM and in one pigeon 100% of the membranes was covered with tissue adhesive. The adhesive makes the membranes rigid and does not allow any vibratory movements of the covered parts. Some tissue adhesive also covered the bronchial rings around the MTM.

All of the pigeons were able to vocalize after the application of tissue adhesive to the MTM. The temporal and frequency patterns of both the trill and the coo parts of the display coo remained intact, even in pigeon 3, in which 100% of the MTM had been covered. However, the post-operative coos were generally softer, and the distribution of energy across the harmonic spectrum changed. In pre-operatively recorded vocalizations, the second harmonic was most prominent and the fundamental frequency was strongly suppressed, whereas the first harmonic contained most energy after the MTM had been covered (Fig. 11). The possible role of the MTM in influencing the harmonic structure will be treated elsewhere (F. Goller, O. L. Larsen and R. A. Suthers, unpublished observations).

Discussion

Biomechanical effects of the syringeal muscles

The endoscopic evidence largely confirms the results of earlier studies regarding the biomechanical effects of the contraction of the syringeal muscles. Contraction of the TL compresses the tracheal segment situated between the insertion points and exerts a pull directly on the LTM, drawing it rostral externally and hence withdrawing it from the syringeal lumen. This abductive effect can be substantial during maximal contraction, but is fairly weak during normal quiet respiration when TL activity is associated with the expiratory phase. This role of reducing syringeal resistance to airflow agrees well with the proposed action of TL contraction in a number of non-passerines. It is interesting that, despite the large differences in syrinx morphology of different bird taxa, the TL, where present, is always involved in opening the airways. This can be a direct withdrawal of the LTM, as in the case of pigeons and doves (Warner, 1972; Ballintijn *et al.* 1995), or an indirect effect by rotation of a syringeal or bronchial cartilage which causes abduction of the LTM in chickens, swiftlets *Collocalia spodiopygia* and oilbirds *Steatornis caripensis* (Gaunt and Gaunt, 1977; Suthers and Hector, 1982, 1985). Even in songbirds, where a number of intrinsic muscles are responsible for the mechanical control of sound production, the main

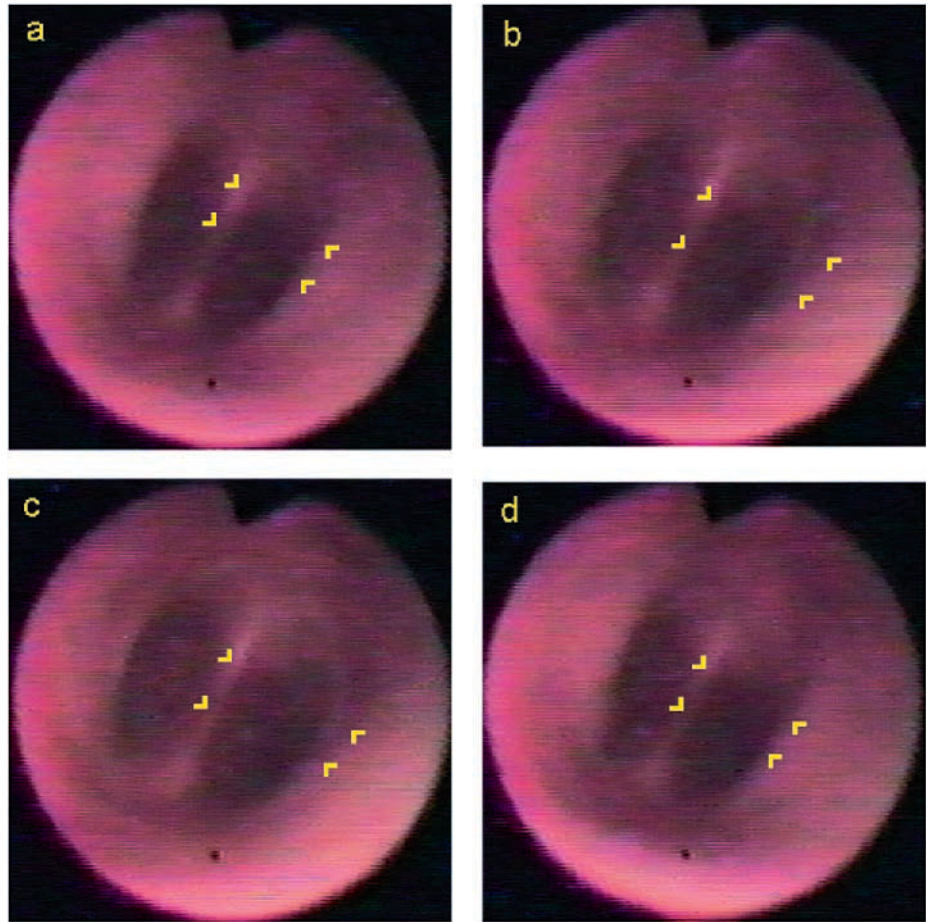


Fig. 5. Inside top view through the angioscope of the syrinx before (a), during (b,c) and after (d) maximal *m. tracheolateralis* (TL) stimulation indicating maximal abduction. These frames are taken from the same individual as those in Fig. 3, but are from a later stage in the experiment and filmed with a slightly greater distance between the syrinx and angioscope lens. Note that the syrinx is in a more abducted state before (a) and after (d) the stimulus than the normal resting position in Fig. 3a,d. Orientation and arrows as in Fig. 3.

abductor, the *m. tracheobronchialis ventralis*, is a morphological continuation of the ventral portion of the TL muscle and both appear to be functionally related (Goller and Suthers, 1996).

In pigeons, ST contraction draws the trachea caudad and (by moving the first two tracheal rings closer to each other) causes folding of the LTM into the tracheal lumen. However, maximal shortening of ST does not cause full closure of the airways in our experiments. Even though the positioning of the birds on

their back may have had some effect on the extent of adduction achieved, it is likely that full closure of the syrinx cannot be achieved in pigeons by active adduction (i.e. ST contraction) but requires increased air-sac pressure. In other non-passerines, ST also appears to serve an adductive role, but whether ST contraction can fully close the syrinx without simultaneously increased air-sac pressure cannot be determined from the available physiological data (Gaunt and Gaunt, 1977; Suthers and Hector, 1982, 1985). Nevertheless, the extent of active

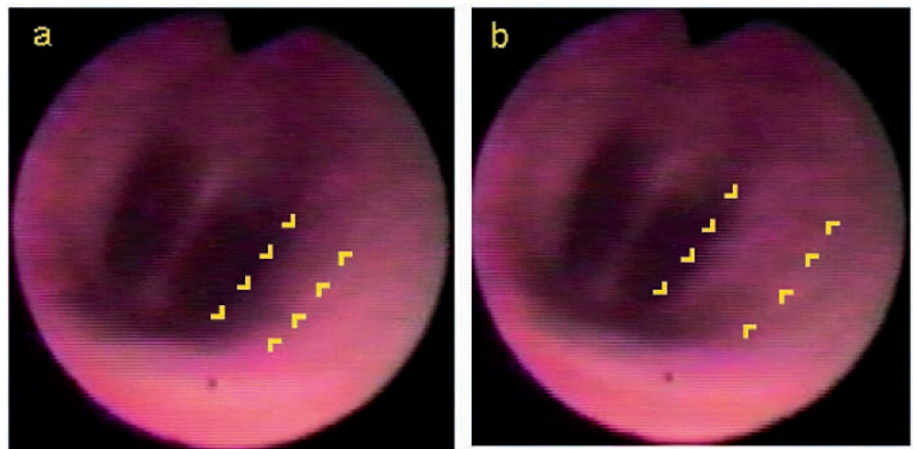


Fig. 6. Inside top view of the syrinx before (a) and during (b) maximal stimulation of *m. sternotrachealis* (ST) indicating maximal active adduction. Orientation and arrows as in Fig. 3.

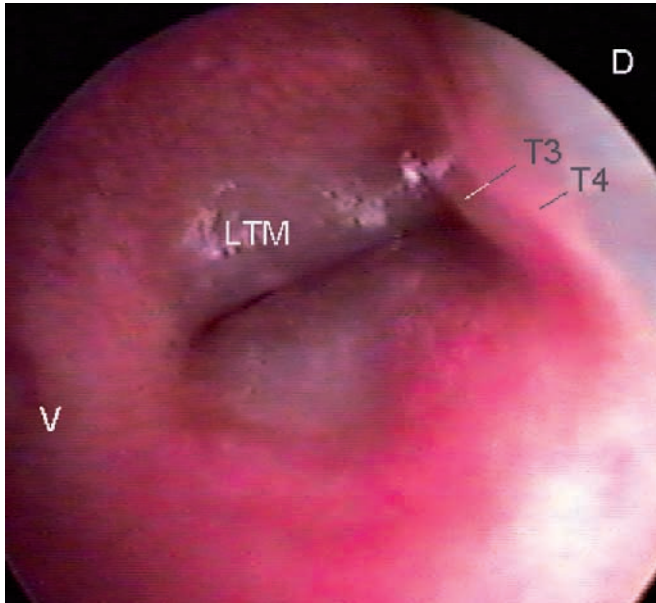


Fig. 7. Air-induced phonatory position shown in a top internal view of the syrinx through the arthroscope. The lateral tympaniform membranes (LTM) are bulged in a rostral direction and form a narrow slot. Note also the deformed dorsal wall of the trachea. T3, T4, tracheal rings 3 and 4. Other abbreviations as in Fig. 3.

adduction and thus the role of ST during phonation may differ markedly between different species and between different vocalizations of one species. In chickens and doves, airflow and pressure recordings indicate that some vocalizations are generated with high syringeal resistance and with relatively low subsyringeal air-sac pressures, suggesting that active adduction contributes, whereas others are generated with very low syringeal resistance and high rates of airflow (Brackenbury, 1980; Gaunt *et al.* 1976). In oilbirds and swiftlets, syringeal resistance is generally higher than in chickens during the production of echolocation clicks, and the syrinx is fully adducted by active means between double clicks (Suthers and Hector, 1982, 1985). In songbirds, the main adductor muscles are the dorsal intrinsic muscles, which can actively close the syrinx during normal respiration as well as in conjunction with high phonatory pressures, and the precise role of ST contraction is not clear (Goller and Suthers, 1996).

Sound production

Understanding the full range of biomechanical action of the syringeal muscles allows us to interpret syringeal configuration during air-induced phonation and to assess its relevance for mechanisms of sound generation during natural vocalization.

Increased subsyringeal air-sac pressure passively adducts

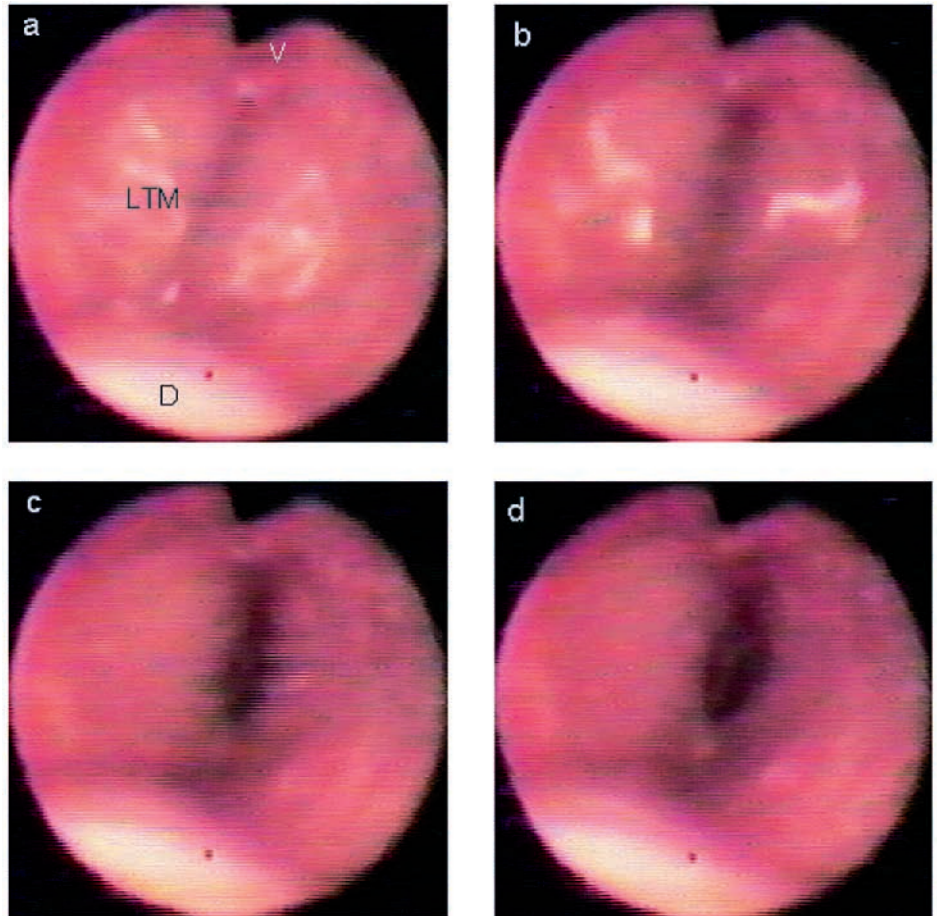


Fig. 8. Internal top angioscopic view of the syrinx during air-induced phonation before (a) and during strong m. tracheolateralis (TL) stimulation (b–d) showing abduction in consecutive frames. As the syringeal lumen becomes larger in diameter (c), vibrations stop (d). Orientation and abbreviations as in Fig. 3.

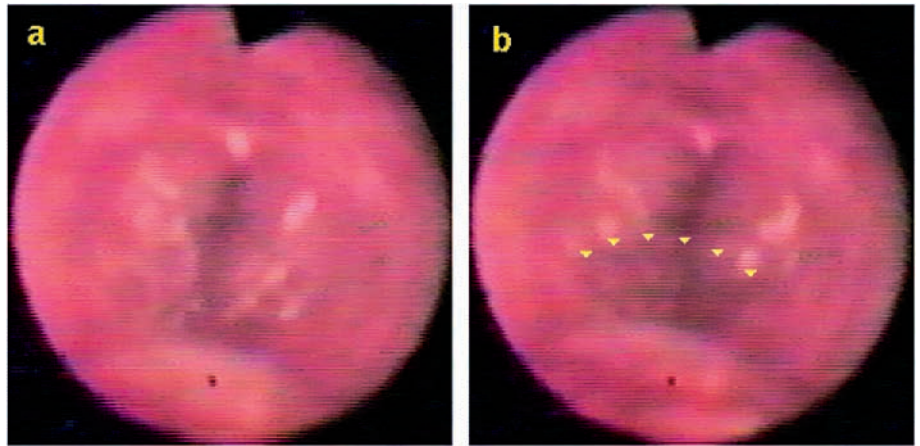


Fig. 9. Internal top view of the syrinx during air-induced phonation before (a) and during (b) weak stimulation of the left m. tracheolateralis (TL) muscle showing asymmetrical changes in the geometry of the fold of the lateral tympaniform membranes. The dorsal area is drawn caudad, changing the light reflection pattern (yellow arrowheads enclose the affected area of the lateral tympaniform membrane). Orientation as in Fig. 3.

the LTM by moving the membranes rostrad and medial. Thus, the membranes bulge in the cranial direction, forming folds (Fig. 12). The exact geometry of the membrane fold (for example, how close its rostral and caudal sides approach each other) is not known, but may be under the control of the syringeal muscles as well as influenced by pressure and the rate of airflow. Once the LTM are fully adducted, vibrations of the membranes start at a given flow rate, resulting in sound generation. Air-induced vibratory movements are complex and so is the acoustic structure of the sound generated. The airspace of the invaginated external part of the fold is continuous with the interclavicular air sac, which may be an important feature determining its vibratory movements. The vibratory behavior of these ballooning membrane folds can be expected to be quite different from that of mammalian vocal folds, which are solid masses of muscle tissue covered by a tightly adherent mucous membrane (Zemlin, 1968).

The phonatory position implied in Fig. 12 and the vibratory behavior of the LTM suggest that air-induced sound is produced by a mechanical mechanism. Elevated pressure causes complete adduction and folding in the rostrad direction of the membranes, thereby increasing syringeal resistance. This higher resistance leads to a higher velocity of airflow and the generation of a local pressure differential (the Bernoulli effect) which, in balance with elastic forces, causes vibrations of the membrane folds.

It is difficult to assess from the present results which of the two mechanical models ('classical' versus 'pulse tone') describes air-induced phonation better. The phonatory position and the fact that close contact and vibratory activity of the LTM are required for sound production suggest that the actual sound-generating mechanism follows the 'pulse tone' model. During air-induced phonation, the vibrating membrane folds appear to approach each other, thus closing the syringeal lumen. However, the resolution of the video equipment does not allow us to determine whether full closure is really achieved during every vibratory cycle. It is therefore possible that the actual vocal mechanism is intermediate between the compression-rarefaction and the pulsatile model (Dürrewang, 1974; Gaunt and Gaunt, 1985).

The second model of avian phonation, the 'aerodynamic hypothesis', suggests that an aerodynamic mechanism is responsible for the generation of pure tones. In this model, sound is generated by high-velocity airflow through one or two constrictions, which produces a series of vortices similar to the sound-generating mechanisms of a hole-tone whistle (Gaunt *et al.* 1982; Casey and Gaunt, 1985; Nottebohm, 1976). Gaunt *et al.* (1982) suggested the junction between the primary bronchi and the trachea as a possible site of constriction in *S. risoria* but, because of anatomical differences, this constricting mechanism cannot occur in other Columbidae (Warner, 1972; Ballintijn *et al.* 1995). The adducted LTM form a slot and could, therefore, also function as such a constriction. However, our observation that audible sound was present only when the LTM vibrated poses a problem for the aerodynamic hypothesis. Sound was never generated when the LTM were in the adducted phonatory

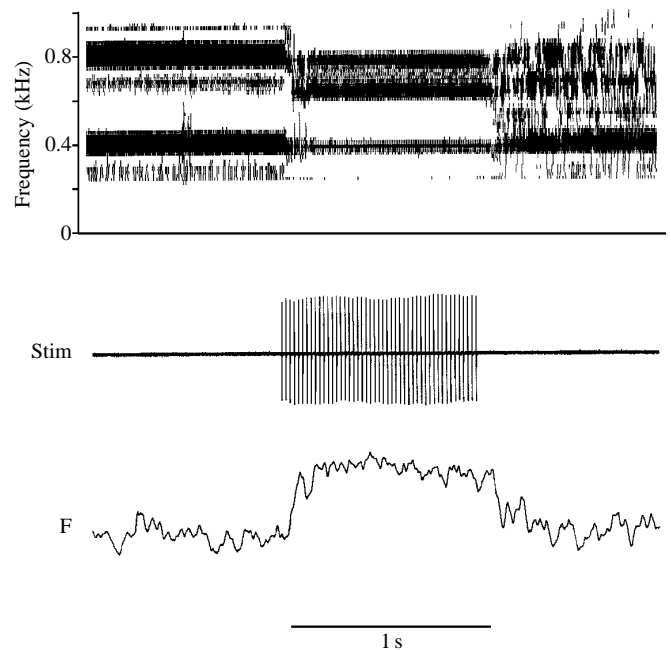


Fig. 10. Air-induced sound changes in complex ways in response to m. tracheolateralis (TL) stimulation (spectrogram top). Tracheal airflow (F) increases during TL stimulation (Stim, stimulus monitor).

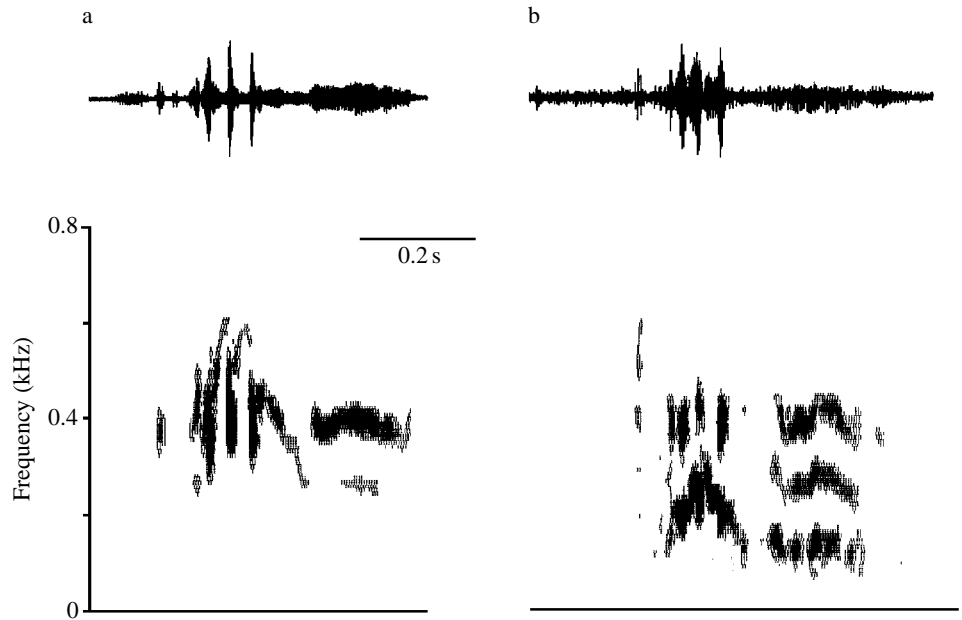


Fig. 11. Display coo of an intact pigeon before (a) and after (b) 100% of the medial tympaniform membranes (MTM) had been covered with tissue adhesive in the same individual. The frequency and amplitude pattern (top, oscillographic, and bottom, spectrographic representation) of the coo remain intact, but the coo is softer and its fundamental frequency is much more prominent after the MTM has been disabled.

position but not vibrating or when vibrations were stopped by stimulation of TL or by approaching the slot with the angioscope. This strongly suggests that sound is generated by vibrations of the folded LTM and not by a whistle mechanism. Similarly, in spontaneously vocalizing pigeons, display coos do not appear to be produced by a whistle mechanism. These vocalizations remain intact after implantation of a flow-straightening device in the trachea to prevent the formation of stable suprasyringeal vortices (Suthers and Zuo, 1991).

How relevant are our findings on air-induced sound generation for natural vocalization?

In order to address this question, we needed to confirm that the LTM are the primary sound generator in spontaneously vocalizing pigeons. Because in pigeons and doves the LTM are an order of magnitude thicker than the MTM, they were not considered to be the sound source. Sound was believed to arise from vibrations of the MTM following the 'classical avian model' (Warner, 1972; Ballintijn *et al.* 1995). We now show clearly that preventing vibration of the MTM neither mutes pigeons nor affects the frequency and amplitude patterns of their vocalizations, suggesting that the MTM are not the primary sound generators. If the LTM are disabled, however, sound production by the excised syrinx is inhibited (Baptista and Abs, 1983), thus confirming the main role of the LTM as sound-generating structures.

One problem for generalizing the sound-production mechanism from air-induced to natural phonation is the fact that the syringeal muscles were not activated in our experiments, but are usually activated during vocalizations. In doves, both syringeal muscles become active shortly before vocalizations start (Gaunt *et al.* 1982). It is therefore necessary to assess the role of the syringeal muscles in combination with elevated air-sac pressure in changing the syringeal configuration for phonation.

During air-induced phonation, pressurization of the air-sac system passively adducts the LTM. This adduction is initiated by small pressures and, as pressure increases, the two membrane folds approach each other more and more closely until, at a given rate of airflow, vibrations start. The pressures required to induce phonation by injecting air tend to be high, but are within the natural range of pressures measured during spontaneous vocalizations in doves (Gaunt *et al.* 1982). Similarly, the pressures needed to generate sounds in freshly dead pigeons are within the natural range and lower than those needed to induce sound production when the syrinx was excised and tested in a model (Abs, 1980). In chickens, the pressures required to induce sound generation are also high but

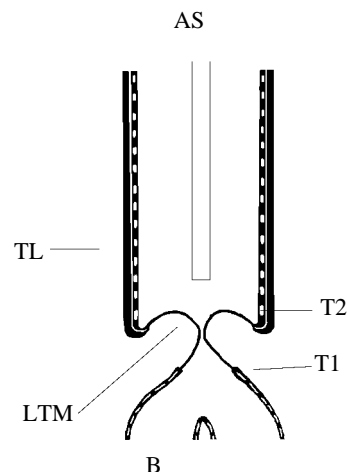


Fig. 12. Schematic drawing of a sagittal section of the air-induced phonatory position of the syrinx. The exact folding of the lateral tympaniform membranes (LTM) is not known. TL, m. tracheolateralis; T1, T2, tracheal rings 1 and 2; B, bronchus; AS, angioscope.

physiologically meaningful (Gaunt *et al.* 1976; Gaunt and Gaunt, 1977).

Syringeal adduction can occur passively by increased subsyringeal pressure and/or actively by contraction of the ST. At normal respiratory pressures, full ST contraction does not result in complete closure of the syringeal lumen, suggesting that the additional passive process must be essential for phonation. The active process, however, does not appear to be required for sound-generation since severing the ST bilaterally in pigeons does not prevent phonation and does not drastically change the acoustic structure of coos (Smith, 1977). The ST may therefore facilitate sound production and add fine control over the biomechanical events. If this is correct, pigeons with disabled ST should generate coos with higher subsyringeal pressures than intact birds. Because the activity of the ST and the effects of posture on the positioning of the trachea may contribute to adduction in spontaneously vocalizing birds, the requirement for higher pressures to induce phonation by injecting or withdrawing air in anesthetized birds is not surprising. This interpretation is consistent with the finding that, in chickens, low-intensity sounds require ST activity but high-intensity sounds do not (Brackenbury, 1980). Whatever the exact role of the ST in pigeons, it is not required for sound generation, supporting the hypothesis that a similar mechanism is used to generate sound during air-induced and natural phonation.

Activity of TL during natural phonation is unlikely to have a major influence on the basic mechanism of sound production. Contraction of the TL withdraws the membranes from the syringeal lumen and, if TL were strongly activated, sound production would be prevented rather than initiated. The role of TL during phonation must therefore be one of fine control of the tension of the LTM and possibly of the geometry of the membrane folds influencing the acoustic structure of the sound. Active withdrawal of the membranes by weak TL stimulation generates amplitude-modulated air-induced sound, and TL may therefore also contribute to amplitude modulation of naturally generated sound (see also Gaunt *et al.* 1982). In pigeons, the fundamental frequency of air-induced sounds is within the range of that of natural vocalizations, and TL stimulation during air-induced phonation changes the acoustic structure of the sound.

On the basis of the preceding discussion, we suggest that the principal sound-generating mechanism for natural phonation in pigeons is close to a 'pulse tone' mechanism, similar to the mechanism described for air-induced phonation. However, given the variety of vocalizations even within the repertoire of pigeons, the possibility that other mechanisms or 'hybrid' mechanisms may also play a role in the generation of some calls cannot be excluded. The possibility that a similar mechanism is used in the more complex songbird syrinx, as suggested for the Indian hill mynah (*Gracula religiosa*) during imitation of human speech (Klatt and Stefanski, 1974), could be tested by endoscopic analysis of the passerine syrinx.

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