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Direct observation of syringeal muscle function in songbirds and a parrot

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Summary

The role of syringeal muscles in controlling the aperture of the avian vocal organ, the syrinx, was evaluated directly for the first time by observing and filming through an endoscope while electrically stimulating different muscle groups of anaesthetised birds.

In songbirds (brown thrashers, *Toxostoma rufum*, and cardinals, *Cardinalis cardinalis*), direct observations of the biomechanical effects of contraction largely confirm the functions of the intrinsic syringeal muscles proposed from indirect studies. Contraction of the dorsal muscles, m. syringealis dorsalis (dS) and m. tracheobronchialis dorsalis, constricts the syringeal lumen and thus reduces airflow by adducting connective tissue masses, the medial (ML) and lateral (LL) labia. Activity of the medial portion of the dS appears to affect the position of the ML and, consequently, plays a previously undescribed role in aperture control. Under the experimental conditions used in this study, full constriction of the syringeal lumen could not be achieved by stimulating adductor muscles. Full closure may require simultaneous activation of extrinsic syringeal muscles or the supine positioning of the bird may have exerted excessive tension on the syrinx. Contraction of m. tracheobronchialis ventralis enlarges the syringeal lumen and thus increases airflow by abducting the LL but does not affect the ML. The largest syringeal muscle, m. syringealis ventralis, plays a minor role, if any, in direct aperture control and thus in gating airflow.

In parrots (cockatiels, *Nymphicus hollandicus*), direct observations show that even during quiet respiration the lateral tympaniform membranes (LTMs) are partially adducted into the tracheal lumen to form a narrow slot. Contraction of the superficial intrinsic muscle, m. syringealis superficialis, adducts the LTMs further into the tracheal lumen but does not close the syringeal aperture fully. The intrinsic deep muscle, m. syringealis profundus, abducts the LTMs through cranio-laterad movement of a paired, protruding half-ring. The weakly developed extrinsic m. sternotrachealis seems to increase tension in the ipsilateral LTM but does not move it in or out of the syringeal lumen.

Key words: brain stimulation, brown thrasher, *Toxostoma rufum*, cockatiel, *Nymphicus hollandicus*, endoscopic analysis, muscle stimulation, Northern cardinal, *Cardinalis cardinalis*, syrinx.

Introduction

Early research revealed that the vocal organ of birds, the syrinx, is a distinct differentiated part of the airway. Unlike the human larynx, the bird syrinx is positioned inside an air sac close to the lungs, where the windpipe, the trachea, bifurcates into the two primary bronchi (Du Verney, 1686). Like the human larynx, however, the syrinx consists of specialised cartilaginous structures, connective tissue masses, membranes and a number of muscles. The syringeal muscles are intrinsic or extrinsic. The former originate and insert completely within the syrinx and probably control the position of syringeal elements. The latter originate or insert outside the syrinx and probably affect the syrinx as a whole (Gaunt, 1983).

Avian sound production is commonly believed to be initiated by the formation of a constriction of the airway, the syringeal aperture. Simultaneous muscular compression of air sacs increases subsyringeal air pressure and creates a pressure differential across the syrinx. The pressure differential increases the expired airflow velocity, inducing Bernoulli forces, which act on the tissue forming the constriction (labia or membranes, see below) and pull it towards the centre of the lumen. The labia or membranes are thought to constitute a self-oscillating system, which is driven by opposing Bernoulli forces and elastic recoil forces. This system generates and sustains the tissue vibrations that constrict and enlarge the syringeal aperture, which modulates the airflow like a pneumatic valve and produces the sound in the tracheal column of air (e.g. Larsen and Goller, 1999).

The syringeal muscles undoubtedly play an important role in preparation for phonation and in controlling the acoustic properties of emitted sound. Our understanding of the

functional roles of individual muscles, however, is based mainly on investigations of the dissected syrinx in, for example, songbirds (e.g. Miskimen, 1951; Chamberlain et al., 1968) and parrots (e.g. Nottebohm, 1976; King, 1989) and on indirect physiological evidence from electromyographic (EMG) recordings of muscle activity in songbirds (e.g. Vicario, 1991) and parrots (e.g. Gaunt and Gaunt, 1985a) and from simultaneous recordings of airflow, EMG activity and subsyringeal air-sac pressure during spontaneous song in songbirds (Goller and Suthers, 1995, 1996a,b; Suthers and Goller, 1997).

These indirect songbird studies have suggested that some of the intrinsic muscles control the syringeal aperture (see Fig. 1). The dorsal muscles are the main adductors and the m. tracheobronchialis ventralis the main abductor. Their activity correlates with decreasing and increasing airflow through the syrinx, respectively (Goller and Suthers, 1996a). The largest syringeal muscle, m. syringealis ventralis, does not appear to gate airflow. However, it may regulate the tension of the syrinx, since its EMG activity is positively correlated with the fundamental frequency of the sound generated and closely parallels frequency modulation (Goller and Suthers, 1996a). Investigations of the parrot syrinx have suggested that the intrinsic muscles, m. syringealis superficialis and m. syringealis profundus, are arranged as antagonists, whose action narrows and widens the syringeal lumen, respectively (see Fig. 5).

In the present study, we video-filmed the interior of the syrinx through an endoscope while electrically stimulating individual syringeal muscles or the tracheosyringeal nerves, which innervate the syringeal muscles, to investigate the biomechanical effects of syringeal muscles directly. In addition, we filmed the phonating syrinx of brain-stimulated songbirds to visualise complex muscle action during coordinated syringeal movements.

Materials and methods

Acute experiments were performed at the Biomedical Laboratory, Odense University Hospital, Denmark, with permission from the Danish Animal Experimentation Inspectorate and at Myers Hall, Indiana University in Bloomington, Indiana, following federal regulations for animal experimentation. Three male cockatiels (*Nymphicus hollandicus*) were acquired from a local vendor in Odense. These birds were anaesthetised 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 birds deeply anaesthetised). At the end of experiments, the birds were killed with an overdose of anaesthetic, and the syrinx was dissected and photographed to document syringeal morphology. At Indiana University, the same procedure was followed for three female cardinals (*Cardinalis cardinalis*) and two male brown thrashers (*Toxostoma rufum*). Songbirds were anaesthetised with chloropent (initial intramuscular injections of 3.6 µl g^{-1} for cardinals and 3.9 µl g^{-1} for thrashers).

Muscle and nerve stimulation

Birds were positioned on their back, and the interclavicular air sac was opened to expose the intrinsic syringeal muscles (see Figs 1, 5). In cockatiels, the interclavicular air sac was accessed only after carefully dissecting the caudal end of the crop free and pushing it to the side. Bipolar electrodes (songbirds, insulated stainless-steel wire, 0.025 mm in diameter; cockatiels, Teflon-coated silver wire, 0.075 mm in diameter) were inserted unilaterally into different muscles near their rostral insertion. A stimulator unit (Grass Instruments, type S88 with SIU5 isolation unit or DISA, type 14E11) delivered electrical pulse trains. Pulse duration was 1 ms, intensity varied between 0.5 and 1.0 mA and pulse trains 0.4–1 s in duration were delivered at 100 Hz.

In songbirds, we stimulated only the intrinsic syringeal muscles and not the smaller extrinsic muscles (the antagonists m. sternotrachealis and m. tracheolateralis). Electrode placement paralleled recording electrode sites in Goller and Suthers (1996a). In cockatiels, the m. syringealis superficialis was stimulated close to its caudal insertion on the bronchus to avoid simultaneous activation of the closely apposed m. syringealis profundus (see Fig. 5). Stimulation of syringeal muscles took place during quiet respiration. In two individual songbirds, the tracheosyringeal branch of the left hypoglossal nerve was resected to eliminate spontaneous respiratory activity, which tended to obscure the muscle-stimulation-induced labial movements on that side.

Anatomical studies on parrots have shown that half-way down the trachea the right tracheosyringealis nerve (Nts) crosses over the ventral surface of the trachea and anastomoses with the left branch. Close to the syrinx, the common anastomosis divides again to innervate both halves of the syrinx (e.g. Nottebohm, 1976). Each half of the budgerigar (*Melopsittacus undulatus*) syrinx receives motor commands from both left and right halves of the hypoglossus nucleus *via* axons crossing over in the anastomosis (Manogue and Nottebohm, 1982; Heaton et al., 1995). Visual inspection showed a similar overall arrangement in cockatiels, and in one bird we stimulated the right branch of NXIIts cranial to the point of crossing over to the left side.

Brain stimulation

Songbirds were placed in a stereotaxic apparatus, and a small opening was made in the skull. Phonation was induced by stimulating the left high vocal centre through bipolar tungsten electrodes (Grass stimulator type S88 with SIU5 isolation unit, pulse duration 1 ms, train duration 1–5 s, pulse repetition rate 60–100 Hz, stimulus intensity 100 µA).

Endoscopic filming of the syrinx

For internal views of the syrinx, the rostral end of the trachea was exposed by an incision in the skin 1–3 cm below the glottis. Here, an opening was cut to facilitate the insertion of

an angiofibrescope into the trachea, and the lens was guided close to the syrinx for internal views (see Figs 1B and 5C). The angiofibrescope (Olympus AF type 14, 1.4 mm outer diameter, 2–50 mm depth of field, 75 ° field of view or Olympus AF type 22A, 2.2 mm outer diameter, 2–50 mm depth of field, 75 ° field of view, 120 ° up/down angulation) was connected to an image control unit (Olympus type OTV-A), a 300 W xenon light source (Olympus type CLV-A) and a video monitor (Olympus type OEV141). The syrinx was then filmed (PAL standard, 25 frames s^{-1}, shutter speed 0.02 s) during normal respiration and during electrical stimulation of individual syringeal muscles. Simultaneously, one of the authors monitored the vocal organ from the outside through a microscope under high power to verify contraction of the targeted muscles and to adjust parameters of electrical stimuli. Endoscope output was recorded on a video cassette recorder together with spoken comments (songbirds, Panasonic, type AG-W1-P; parrots, SONY videoHi8, type EVO-9800P). Video segments were digitised (Vincent 601 PCI board and Media100 software, Data Translation Inc.) on a Power Macintosh 9500 (or 7500/100) computer. Single images were imported into software packages (Corel v. 5.0, Adobe PhotoShop v. 5.3 and Adobe Illustrator v. 8.0) for the preparation of figures.

Results

The songbird syrinx

Intrinsic muscle function

Stimulation of the dorsal muscles (electrodes placed in m. tracheobronchialis dorsalis, dTB, and possibly in the underlying lateral portion of m. syringealis dorsalis, dS) (Fig. 1A) adducted both the lateral (LL) and medial (ML) labia into the bronchial lumen (Fig. 2a–d). Adduction of the LL contributes much more to the closing of the syringeal valve than adduction of the ML. However, it was too difficult to quantify the relative contributions of the two labia from the two-dimensional representation of a complex three-dimensional movement. Full closure of the bronchial lumen could not be achieved by stimulating the dorsal muscles even

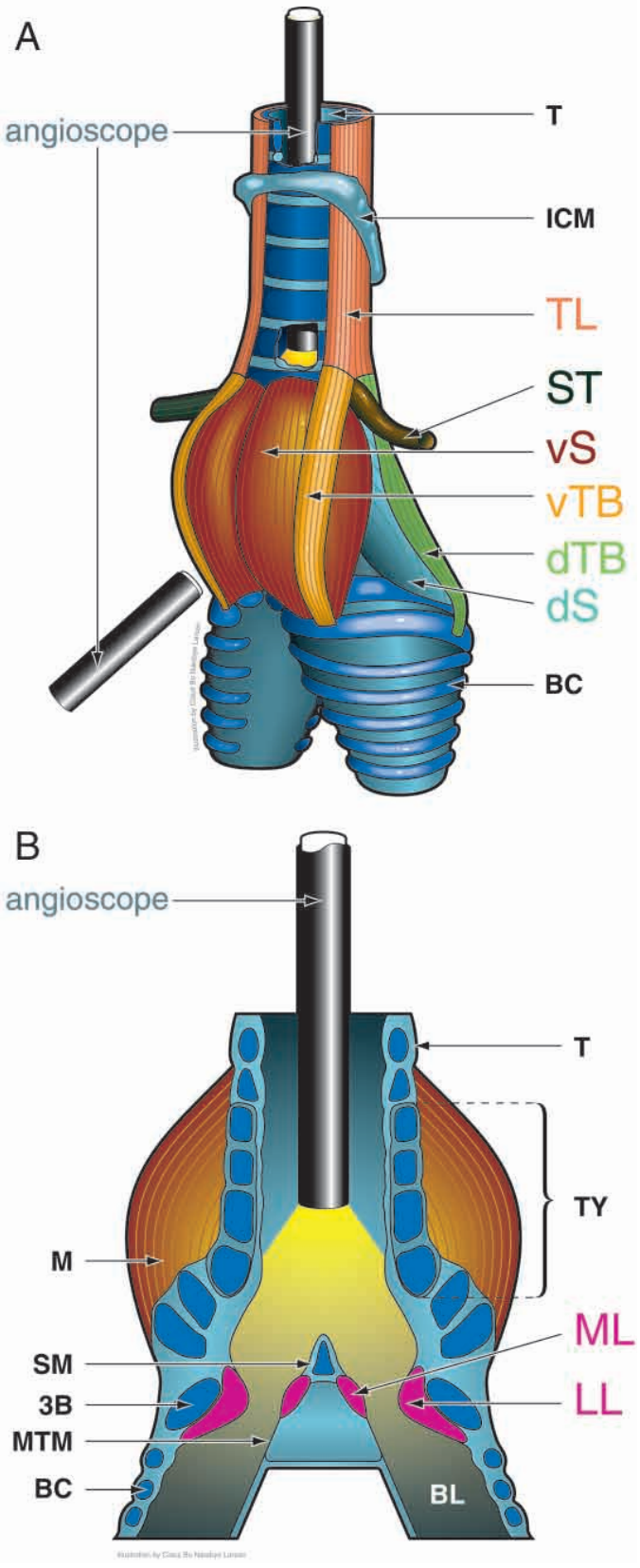

Fig. 1. The songbird tracheobronchial syrinx is a complex bipartite structure situated where the connecting tubes to the lungs (the bronchi) join the windpipe (the trachea). (A) Schematic ventro-lateral external view of a songbird syrinx depicting the syringeal muscles. To illustrate the position of the angioscope used for internal views, parts of the trachea have been removed; the yellow colour indicates endoscope light. The external angioscope indicates the viewing angle in Fig. 4. (B) Schematic horizontal section through a songbird syrinx illustrating the two labial sound sources and the approximate position and field of view of the angioscope (yellow light) in Fig. 3. (For the syringeal views in Fig. 2, the angioscope was advanced towards the left side of the syrinx.) BC, bronchial cartilage; 3B, third bronchial cartilage; BL, bronchial lumen; dS, m. syringealis dorsalis; dTB, m. tracheobronchialis dorsalis; ICM, membrane of the interclavicular air sac; LL, lateral labium; M, syringeal muscle; ML, median labium; MTM, median tympaniform membrane; SM, semilunar membrane; ST, m. sternotrachealis; T, trachea; TL, m. tracheolateralis; TY, tympanum (consisting of four closely apposed or fused tracheosyringeal cartilages); vS, m. syringealis ventralis; vTB, m. tracheobronchialis ventralis.

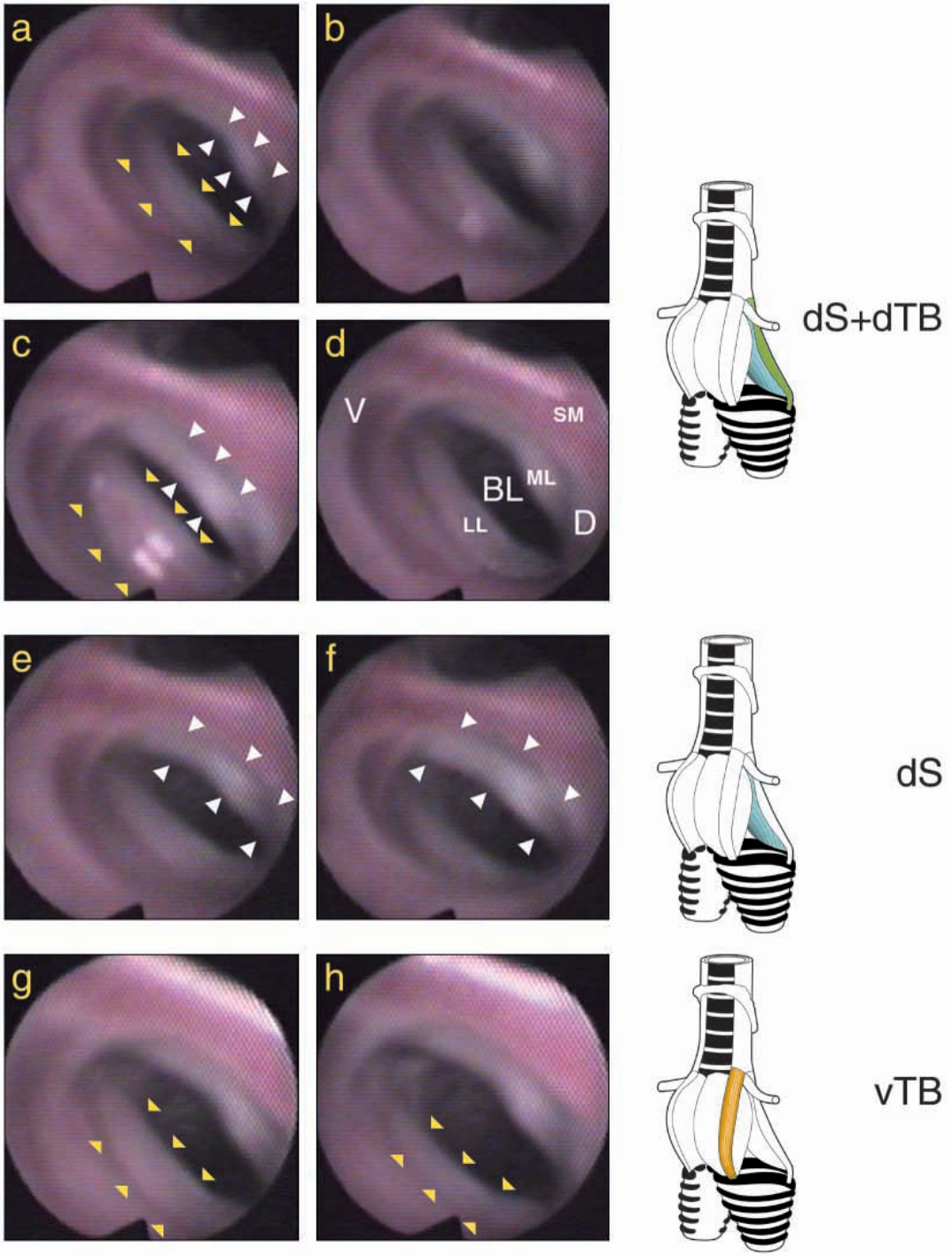

Fig. 2. Internal view through the trachea of the left side of the cardinal syrinx illustrating the effect of electrical stimulation of the ipsilateral muscles shown in the insets to the right. (a–d) Simultaneous stimulation of the m. tracheobronchialis dorsalis (dTB) and m. syringealis dorsalis (dS). In the respiratory position (a,d), the medial (ML) and lateral (LL) labia do not protrude far into the bronchial lumen (BL). Upon strong stimulation, they are adducted (move into the BL), but do not touch and close the aperture (b,c). The arrowheads outline the visible portions of the ML (white) and LL (yellow) and indicate labial extension into the bronchial lumen. When stimulation is terminated, the labia move back into the respiratory position. (e,f) Stimulation of the medial portion of the m. syringealis dorsalis (dS) alone mainly affects the ML, which is moved slightly into the lumen. White arrowheads indicate the change from the respiratory (e) to the stimulated (f) position. dS contraction may also result in a rostro-caudal stretching of the ML suggested by a change in the light reflection pattern in the video. (g,h) Stimulation of the left m. tracheobronchialis ventralis (vTB) causes abduction (movement out of the bronchial lumen) of the left LL but does not noticeably affect the position of the ML. In comparison with the opening before stimulation, the aperture of the bronchus is increased by movement of the LL in the caudo-lateral direction (downwards) in h. D, dorsal side; V, ventral side. SM, semilunar membrane.

with the highest stimulus intensity. Because of the close physical proximity of the two dorsal muscles, they always contracted in concert upon stimulation. This was confirmed by observing twitches under the external microscope. To determine the specific roles of the two dorsal muscles, we placed stimulus electrodes into the medial part of the dS, where there is no physical contact with the dTB. This required dissecting the connective tissue, which attaches the syrinx to the dorsal body wall. Stimulation of the medial part of the dS resulted in a slight adduction of the ML into the bronchial lumen (this movement is obvious in the video but difficult to illustrate in two-dimensional still pictures) (see Fig. 2e,f). Stimulus intensities had to be kept low to avoid simultaneous dTB contraction. A closer examination of the change in the pattern of light reflection suggested not only that the ML was adducted into the lumen but also that it was stretched rostro-caudally.

Contraction of the ventral tracheobronchial muscle (vTB) increased the syringeal aperture by withdrawing the LL from its respiratory resting position in the bronchial lumen. There was no visible effect on the position of the ML (Fig. 2g,h). However, stimulus intensities were kept low to avoid contraction of the underlying portion of the m. syringealis ventralis (vS). A small abduction of the LL also occurred spontaneously during the expiratory phase of respiration. This abduction coincides with weak EMG activity in the vTB (F. Goller, unpublished observations). Spontaneous abduction of the LL was even observed during sustained electrical stimulation of the dorsal muscles, suggesting that activation of the vTB overrides adductive forces.

The exact mechanical events during abduction and adduction of the LL are difficult to ascertain from the endoscopic data. It is obvious, however, that adduction and abduction are achieved by medio-rostral movement (right and up in Fig. 2a–c) of the LL and by latero-caudad movement (left and down in Fig. 2g,h) of the LL. This movement has in the past been ascribed to a rotation of the third bronchial ring (Miskimen, 1951; Chamberlain et al., 1968). When filmed from the outside, however, it becomes obvious that adduction and abduction are not the result of a simple rotational movement by the third bronchial ring. The valving action of the LL constitutes a rather more complicated repositioning, including lateral movement and a slight rotation. Details of the biomechanical events mediating the movement of the ML are even less clear (Goller and Larsen, 1997b).

Stimulation of the largest muscle of the syrinx, the vS, did not affect the syringeal aperture by lateral movement of either labium (Fig. 3). However, it caused a change in light reflection of the ML, which could be interpreted as movement parallel to the long axis of the angioscope. This postulated movement would change the tension of the ML and the attached medial tympaniform membranes (MTMs) (Fig. 1B).

Syringeal movement during phonation

During phonation, the syrinx closes fully (Goller and Larsen, 1997b), but direct stimulation of individual dorsal muscles did not achieve the same degree of adduction. To explore whether additional processes might contribute to the closure of the syringeal valve during phonation, we placed the angioscope such that we viewed the bronchidesmal area directly (lower left angioscope in Fig. 1A) and filmed it during brain-stimulation-induced vocalisations. Sound production was always initiated by a vigorous rostrad movement of the syrinx (Fig. 4) and occurred only when the syrinx was in its most rostral position. The magnitude of this movement is exemplified by the length change of the MTMs, which are stretched to more than double their resting length. Stretching altered the three-dimensional arrangement of the cartilages involved in regulating the syringeal valve.

These observations suggest that stretching of the syrinx in itself contributes to labial adduction by affecting the position of those bronchial rings that are involved in labial movement. We tested this by severing the trachea above the syrinx to view the labia through the external microscope. A gentle cranial pull on the trachea caused distinct adduction of both the LL and ML. This suggests that the three-dimensional suspension of the

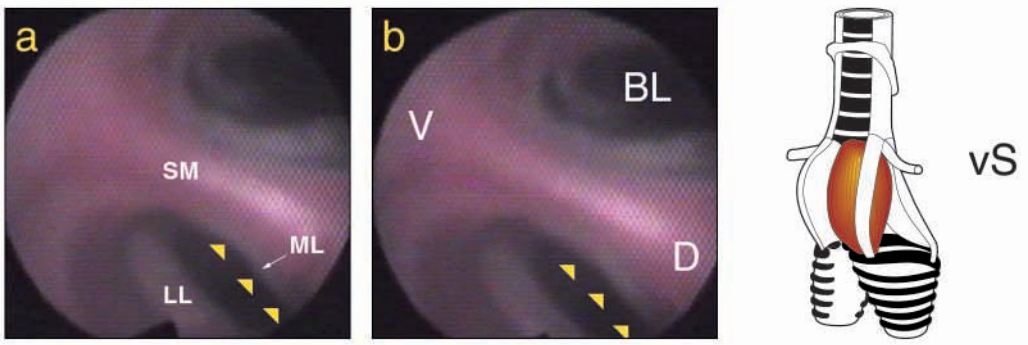

Fig. 3. Electrical stimulation of m. syringealis ventralis (vS), the largest syringeal muscle in songbirds, does not noticeably change the aperture of the bronchus from the respiratory (a) to the stimulated (b) position. However, upon stimulation, a twitching motion of the medial labium is apparent in the video, suggesting that stretching of the medial labium along the rostro-caudal axis may occur (yellow arrowheads). Angioscope position approximately as illustrated in Fig. 1B. D, dorsal side; V, ventral side; BL, bronchial lumen; LL, lateral labium; ML, median labium; SM, semilunar membrane.

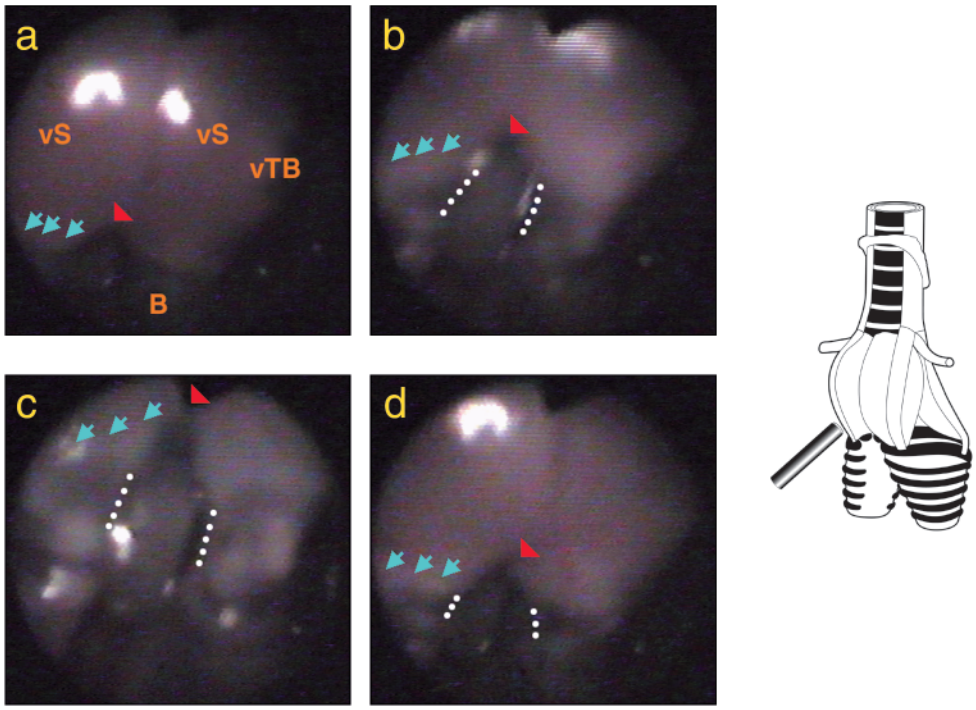

Fig. 4. Sequence of ventral outside views of the brown thrasher syrinx during the transition from quiet respiration (a), *via* reconfiguration in preparation for phonation (b), to phonation (c) and return to the starting point (d) induced by electrical brain stimulation. The sequence illustrates the striking rostrad movement during sound production. To illustrate the movement, red arrowheads mark the caudal end of the syringeal midline and blue arrowheads outline the third bronchial cartilage. The movement results in significant stretching of connective tissue in the bronchial section, including the medial tympaniform membranes (the white dotted line outlines the medial edges of the median tympaniform membrane). B, bronchial cartilage; vS, m. syringealis ventralis; vTB, m. tracheobronchialis ventralis.

bronchial rings, some of which are specially shaped and protrude dorsally from the tube-like bronchi, will in itself produce some adductive movement when the syrinx is stretched.

The cockatiel syrinx

Morphology

Since, to our knowledge, the syrinx of the cockatiel has not been described previously in detail and differs somewhat from published descriptions of the syrinx in other Psittaciformes, we include a section on morphology following the nomenclature of King (1989). In the cockatiel syrinx, we observed and stimulated three pairs of muscles: the extrinsic m. sternotrachealis (ST) and the intrinsic m. syringealis superficialis (SS) and m. syringealis profundus (SP) (Fig. 5). M. tracheolateralis (TL) is a very thin muscle, and its caudal insertion point could not be determined accurately (as in some other parrots) (Gaunt and Gaunt, 1985a; King, 1989).

Each ST attaches cranially to the lateral wall of the trachea, forming a well-developed muscle. At tracheal rings 6–8, the ST transforms into a thin tendon, which passes ventro-laterally over the SS, attaching *en passant* to the ventro-medial parts of tracheosyringeal cartilages 11–14 (King, 1989). Multiple sheets of connective tissue attach the ST to adjacent blood vessels and to the sternal area.

The intrinsic muscles, SS and SP, are closely apposed (Fig. 5A,C). The SS attaches cranially to the dorso-lateral sides of the tympanum (King, 1989), formed by fusion of 4–5 ossified tracheosyringeal cartilages. From here, the SS arches latero-caudally to insert on tracheosyringeal cartilages 10–12.

The cockatiel trachea is markedly constricted craniad to the bronchial bifurcation, as is characteristic for the syrinx in Psittaciformes (e.g. King, 1989). Endoscopic views of the syrinx through the trachea further suggest that the LTMs are folded into the tracheal lumen along their dorso-ventral axis (Figs 5C, 6), which is the result of the close proximity of the cartilages on which they insert. The cranial edge of each LTM is defined by a paired ossified half-ring structure that protrudes dorsally and ventrally from the tube-like trachea to form a dorsal and a ventral protrusion (PP in Figs 5 and 6g–i). Each of these structures is cranially hinged to the ossified tympanum by a highly flexible ligament. The deep syringeal muscle, SP, is partly hidden underneath the SS. The SP attaches cranially to the lateral side of the caudal part of the tympanum while, caudally, it inserts along most of the length of the ipsilateral PP (see Fig. 5B,C). This arrangement gives the SP the shape of an isosceles triangle with the obtuse angle at the tympanum and the acute angles at the dorsal and ventral protrusions.

Syringeal muscle function

It was difficult to place the stimulating electrodes in the SS such that only this muscle was activated. When electrodes were placed near the syringeal insertion point on the tympanum (see Fig. 5C), both the SS and the SP contracted. The result was an initial adductive movement of the ipsilateral LTM, which was immediately overridden by abduction. When the electrodes were placed in the SS near its bronchial insertion (i.e. farthest from the SP), stimulation caused contraction of the SS without also activating the SP. Contractions of the SS resulted in sustained adduction of the ipsilateral LTM (Fig. 6a–c). Adductive movement was more pronounced on the dorsal part of the slot formed by the LTMs, such that at lower stimulus

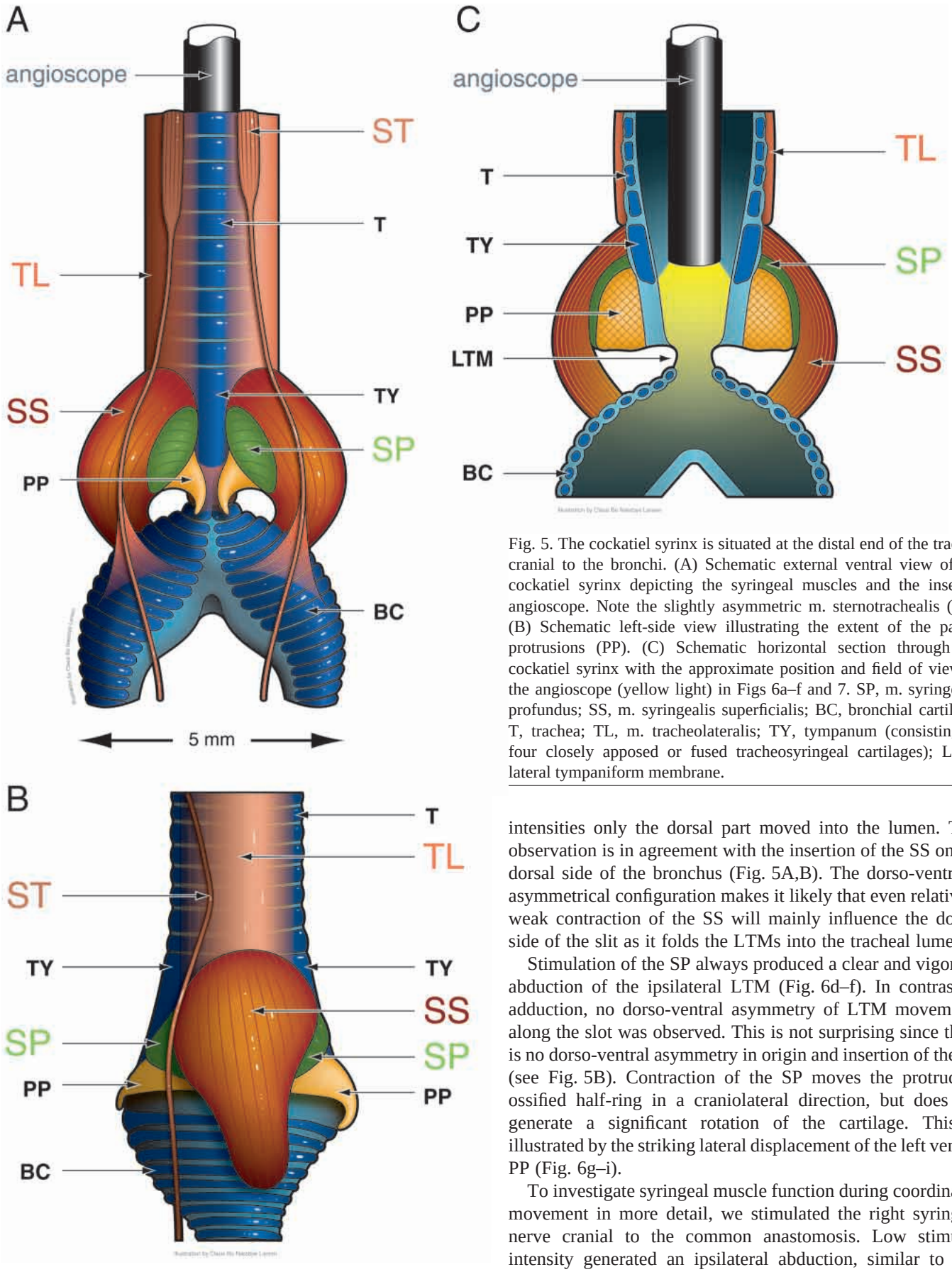

Fig. 5. The cockatiel syrinx is situated at the distal end of the trachea cranial to the bronchi. (A) Schematic external ventral view of the cockatiel syrinx depicting the syringeal muscles and the inserted angioscope. Note the slightly asymmetric m. sternotrachealis (ST). (B) Schematic left-side view illustrating the extent of the paired protrusions (PP). (C) Schematic horizontal section through the cockatiel syrinx with the approximate position and field of view of the angioscope (yellow light) in Figs 6a–f and 7. SP, m. syringealis profundus; SS, m. syringealis superficialis; BC, bronchial cartilage; T, trachea; TL, m. tracheolateralis; TY, tympanum (consisting of four closely apposed or fused tracheosyringeal cartilages); LTM, lateral tympaniform membrane.

intensities only the dorsal part moved into the lumen. This observation is in agreement with the insertion of the SS on the dorsal side of the bronchus (Fig. 5A,B). The dorso-ventrally asymmetrical configuration makes it likely that even relatively weak contraction of the SS will mainly influence the dorsal side of the slit as it folds the LTMs into the tracheal lumen.

Stimulation of the SP always produced a clear and vigorous abduction of the ipsilateral LTM (Fig. 6d–f). In contrast to adduction, no dorso-ventral asymmetry of LTM movements along the slot was observed. This is not surprising since there is no dorso-ventral asymmetry in origin and insertion of the SP (see Fig. 5B). Contraction of the SP moves the protruding ossified half-ring in a craniolateral direction, but does not generate a significant rotation of the cartilage. This is illustrated by the striking lateral displacement of the left ventral PP (Fig. 6g–i).

To investigate syringeal muscle function during coordinated movement in more detail, we stimulated the right syringeal nerve cranial to the common anastomosis. Low stimulus intensity generated an ipsilateral abduction, similar to that

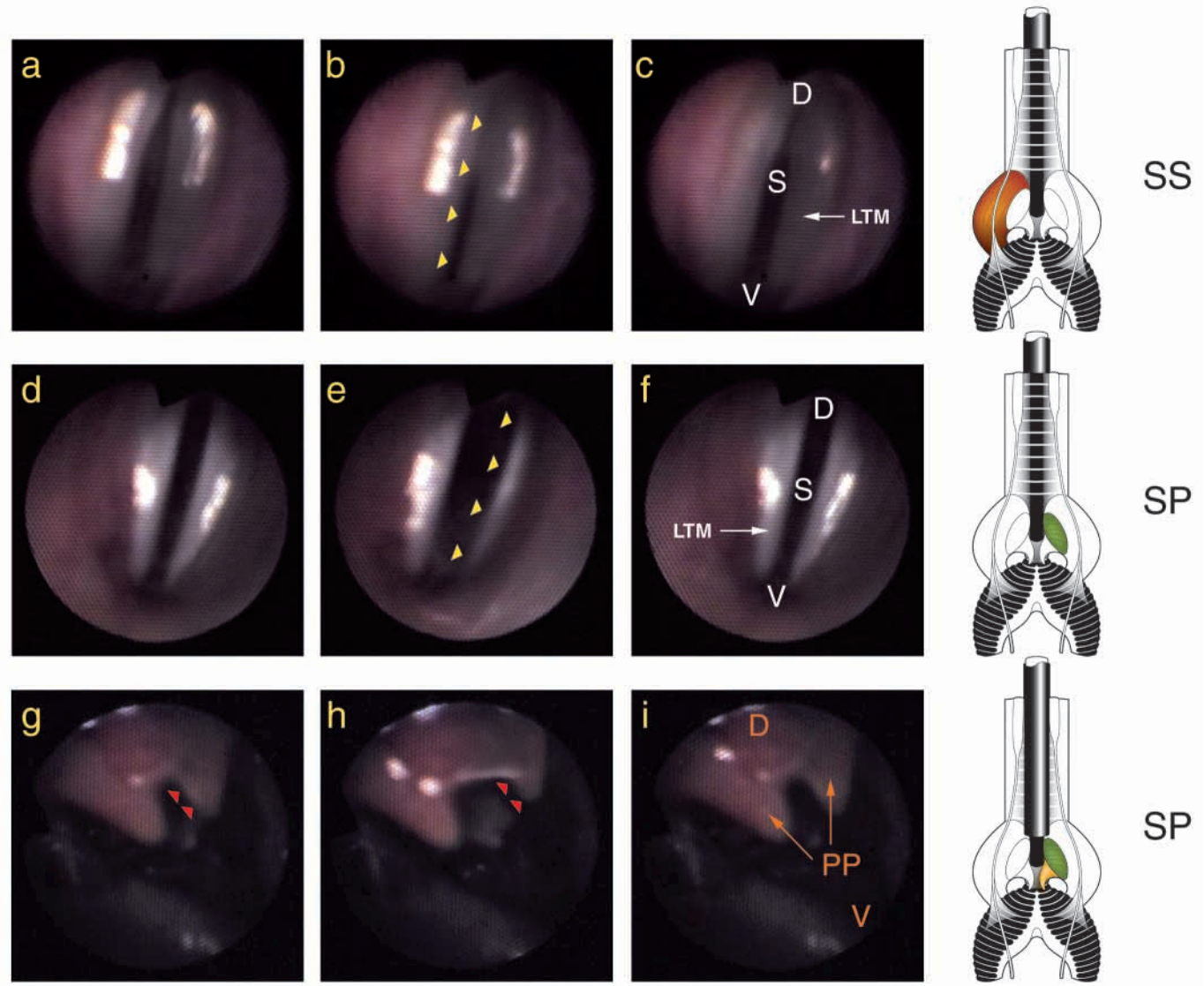

Fig. 6. Internal view through the trachea (a–f) and external view along the trachea (g–i) of the cockatiel syrinx illustrating the effects of electrical stimulation of the muscles shown in the insets to the right. (a,c,d,f) During quiet respiration, the cranially arching lateral tympaniform membranes (LTMs) of the cockatiel syrinx are positioned in the tracheal lumen, forming a dorso-ventral slot (S). (b) Stimulation of the right m. syringealis superficialis (SS) adducts the ipsilateral LTM (left in picture) into the middle of the tracheal lumen, narrowing S (yellow arrowheads). (e) Stimulation of the left m. syringealis profundus (SP) abducts the ipsilateral LTM (right in picture) from the tracheal lumen, widening S (yellow arrowheads). (g–i) The same situation viewed from the outside caudally along the tracheal axis (see inset). Stimulation of the left SP (h) abducts the ipsilateral member of the paired protrusions (PP) (red arrowheads) as it is drawn cranio-laterad. When stimulation is terminated, the left PP move back into the respiratory position (i). D, dorsal side; V, ventral side.

achieved with unilateral SP stimulation. A high-intensity stimulus produced bilateral abduction of the LTMs from the tracheal lumen (Fig. 7). This abductive movement of each LTM substantially exceeded that following SP or weak nerve stimulation.

The function of the m. sternotrachealis (ST) was much more difficult to assess because stimulation did not produce any observable lateral movement of the LTMs. The pattern of light reflection from the LTMs, however, changed slightly upon stimulation, suggesting a possible effect on LTM tension.

Discussion

Functional role of the syringeal muscles

The results of this study, in general, directly confirm interpretations about the functional role of syringeal muscles inferred from studies that are more indirect. The syringeal muscles are instrumental in preparing the syrinx for phonation by moving the labia or lateral tympaniform membranes into the airway and tensing the syrinx appropriately. During phonation, they may modulate the sound generated by varying the constriction and tension of the syrinx.

The main adductors constricting the syringeal aperture are

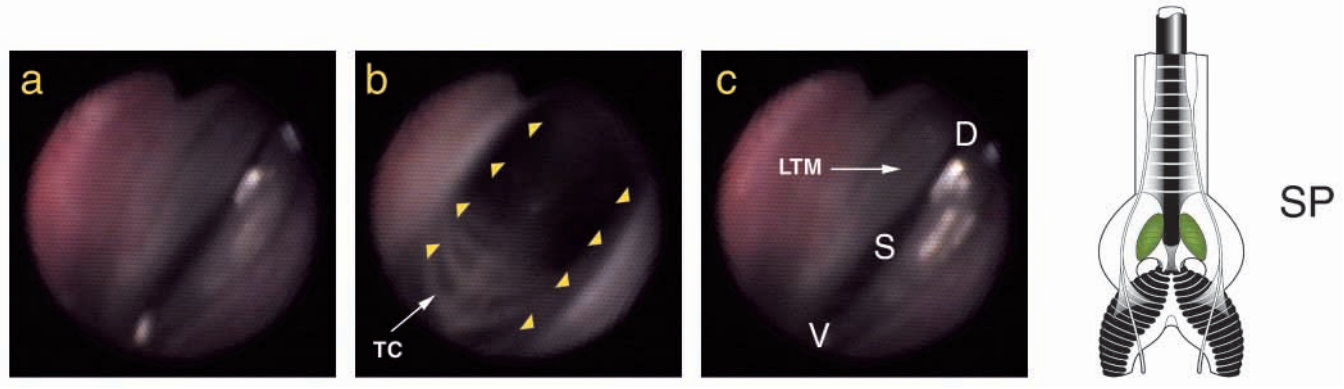

Fig. 7. Electrical stimulation at high intensity of the cockatiel's right tracheosyringeal nerve cranial to the common anastomosis produces a vigorous bilateral abduction of the lateral tympaniform membranes (LTM) (yellow arrowheads). (a) Quiet breathing. (b) Nerve stimulation. Note the four tracheosyringeal cartilages (TC) below the LTM extending towards the bronchial divide. (c) Return to quiet breathing. D, dorsal side; V, ventral side; S, dorso-ventral slot; SP, m. syringealis profundus.

the dorsal muscles, the dS and dTB in songbirds and the SS in the parrot. Enlargement of the syringeal aperture is achieved by contraction of the abductor vTB in songbirds and SP in the parrot. These roles were initially proposed from morphological studies [songbirds (Miskimen, 1951; Chamberlain et al., 1968); parrots (Nottebohm, 1976)] and later indirectly confirmed by EMG studies of these muscles during spontaneous vocalisations [songbirds (Vicario, 1991; Goller and Suthers, 1996a,b); parrots (Gaunt and Gaunt, 1985a)].

EMG studies showed great similarity between the activity patterns of the two dorsal muscles, dS and dTB (Goller and Suthers, 1995, 1996a). EMG electrodes were placed in the dorso-lateral area of the syrinx, where the dTB lies on top of the lateral part of the dS. Because the dorsal part of the syrinx is attached to the dorsal wall, the function of the medial part of the dS was not investigated electromyographically. We now provide evidence that the dTB and the medial part of the dS serve different roles in adduction. Whereas dTB contraction (and possibly contraction of the lateral part of the dS) moves the LL into the syringeal lumen, contraction of the medial part of the dS adducts the ML slightly. Because stimulus intensity had to be kept low to avoid simultaneous dTB contraction, the full biomechanical effects of dS contraction may not have been revealed. In the singing bird, dS activity regulated by the central nervous system may conceivably cause a stronger adduction of the ML. The exact functional role of the lateral part of the dS remains unclear.

Syringeal constriction is physically manifested by movement of both labia into the bronchial lumen. Although, in previous interpretations, adduction was attributed only to the LL (e.g. Brackenbury, 1989), it is now clear that the ML also contributes, albeit to a smaller degree. During phonation, the LL obstructs between 60 and 85 % of the lumen (Goller and Larsen, 1997b), which is similar to the results of direct muscle stimulation in the present study (see Fig. 2a–d).

Our results are also in agreement with earlier studies with respect to the control of the tension of the sound-generating structures. In songbirds, the role of tensing the sound generator and thus of controlling the frequency of the sound generated has been assigned to the vS (Miskimen, 1951). Strong support for this interpretation was derived from the close positive correlation between fundamental frequency and vS EMG amplitude (Goller and Suthers, 1996b). Our endoscopic evidence cannot provide direct evidence for this role. Indirectly, however, it is consistent with this interpretation, because stimulation of the vS changes the light reflection pattern of the ML, which may result from a tensing of the ML. In addition, we now show that contraction of the vS does not affect the position of either labium and, therefore, does not contribute directly to regulation of the syringeal valve, but may do so indirectly by anchoring cartilaginous elements and thus providing counterbalance as other muscles contract.

The specific roles of the extrinsic muscles (ST and TL) in the songbird remain unclear (Goller and Suthers, 1996a,b) and were not tested in this study. However, the observation that the syrinx moves rostrad in preparation for sound production suggests that contraction of the TL in addition to pressurisation of the interclavicular air sac (see Fig. 1A; ICM) may play an important role in mediating this movement (Daley and Goller, 2000). This interpretation predicts increased EMG activity in the TL prior to phonation. Existing EMG recordings from the TL do not support this prediction (Goller and Suthers, 1996a), but they may be problematic because of the uncertain location of electrode placement and the difficulty of determining muscle identity at the border between the TL and the vTB.

In the parrot syrinx, there is no clear relationship between EMG activity of a syringeal muscle and the fundamental frequency of the sound generated, as was found in songbirds (Gaunt and Gaunt, 1985a). Contraction of the SS might indirectly regulate the tension of the LTMs by drawing together the cartilages on which the LTMs insert (Gaunt and Gaunt, 1985a). However, in contrast to the songbird syrinx, increased contraction of these adductors should decrease membrane tension. Thus, the regulation of tension in the parrot syrinx appears to be more indirect than that in songbirds. Our

data do not contradict this interpretation and, in addition, suggest the ST as a potential tension-controlling muscle.

Biomechanical effects of muscle contraction

Although the syringeal valving action and the structures involved have become evident from these and previous endoscopic observations in songbirds, exactly how muscle contraction forces translate into movement of the labia is still unclear. Visualisation of the lateral and ventral surfaces of the syrinx during adduction shows that movement of the LL is not mediated simply by a rotation of the third bronchial ring (see Fig. 1B), as suggested by Miskimen (1951) and Chamberlain et al. (1968). However, endoscopy alone can give us no further insight into the detailed lever action and the three-dimensional rearrangement of the LL and, similarly, does not allow us to study the mechanical aspects of movement of the ML. The study of these biomechanical aspects will require the application of a combination of various techniques from functional morphology. For instance, it will be interesting to see whether the movement of the ML is mediated by the cartilage tensor, a ventro-medial extension of the second bronchial semi-ring described by Setterwall (1901).

The transmission of muscle length changes to valving action by the LTM appears less complicated in the cockatiel syrinx. The smaller number of syringeal muscles and the more visible mechanical arrangement of cartilages present a less complicated biomechanical system than that of the songbird syrinx. The attachment of the SS on the bronchi suggests that adduction is achieved by shortening the rostro-caudal distance between the two cartilages on which the LTMs insert, thus folding the LTMs into the syringeal lumen (see Fig. 5C) (Nottebohm, 1976; Gaunt and Gaunt, 1985a). As suggested by the present observations, this action is dorsally biased and, in addition, may also be refined by ST activity, similar to the effect of the ST in the simpler syrinx of the pigeon (Goller and Larsen, 1997a).

The characteristic shape of the paired ossified half-ring structures (PP) (Fig. 5) with the well-defined insertion areas of the SP suggested that abduction is achieved by pulling the cartilage laterally (Nottebohm, 1976; Gaunt and Gaunt, 1985a). The extent of the rostro-lateral movement of the ventral (see Fig. 6g–i) protrusions of the cartilage illustrates the swinging motion and confirms earlier interpretations.

The biomechanical events involved in regulating the syringeal valve are distinctly different in the parrot syrinx from those of the songbird syrinx. Adduction is more indirect and may play a less important role in the parrot syrinx because of the more constricted resting position. The syrinx of all parrots is characterised by a narrow constriction of the cartilaginous components (e.g. King, 1989). Our endoscopic pictures suggest that, in addition to the morphological constriction, the LTMs are partially adducted by muscular action in the quiet resting position. It is unlikely that this partial adduction is caused by the unnatural positioning of the cockatiels in our experiments, which would have stretched the trachea and syrinx slightly, resulting in abduction rather than adduction.

Complex interactions between syringeal muscles

The present data and previous EMG analyses (Gaunt and Gaunt, 1985a; Vicario, 1991; Goller and Suthers, 1996a,b) clearly indicate that the actual control of the syrinx results from a complex interaction between muscles in combination with the complicated suspension of the cartilaginous framework upon which they act.

Full adduction could not be achieved by direct muscle stimulation of the presumed adductors in either type of syrinx. It is well documented, however, that the syringeal valve can be fully closed during phonation (e.g. Suthers et al., 1994; Goller and Suthers, 1996a,b; Goller and Larsen, 1997b; Larsen and Goller, 1999). In addition, it can be closed during non-vocal behaviour involving high subsyringeal air-sac pressure, such as defecation and yawning, or during regular quiet respiration (F. Goller, unpublished observations). Our observations during phonation suggest that rostrad movement of the syrinx, presumably through TL contraction and pressurisation of the interclavicular air sac, may contribute to full closure. In addition, even weak action by other syringeal muscles may contribute by changing the lever action of the adductors.

It is also striking that enlargement of the syringeal aperture can override induced constriction, seemingly with ease. Strong sustained stimulation of adductor muscles did not prevent spontaneous abduction of the labia during expiration in songbirds. In cockatiels, nerve stimulation as well as stimulation of both intrinsic muscles resulted in abduction of the LTMs. This suggests that biomechanical facilitation of abduction resulted from selective forces demanding a rapid opening of the airways. In songbirds as well as parrots, rapid abductive action may be important for phonation because it is likely to provide one mechanism for generating amplitude modulation (Goller and Suthers, 1996b; Gaunt and Gaunt, 1985a; Banta Lavenex, 1999). A requirement to keep the airways open during the generation of expiratory pressure seems to be a shared characteristic of all types of vocal organs in birds (Nottebohm, 1971; Youngren et al., 1974; Lockner and Youngren, 1976; Gaunt and Gaunt, 1985b). The activity of abductor muscles during the expiratory, but not the inspiratory, phase of quiet respiration (Youngren et al., 1974; Lockner and Youngren, 1976; Vicario, 1991; Goller and Suthers, 1996a) strongly supports the notion that increased expiratory pressure may draw the LTMs or labia passively into the airways.

In contrast, the mammalian larynx is abducted during the inspiratory phase of quiet breathing (e.g. Brancatisano, 1996). It is interesting that, in quietly breathing birds, the glottal valve, the avian homologue of the mammalian larynx, is also enlarged during inspiration but not during expiration (F. Goller and O. N. Larsen, unpublished observation on anaesthetised pigeons, *Columba livia domestica*). So, in quietly breathing birds, the glottal valve is abducted during inspiration while the syringeal valve is abducted during expiration. This out-of-phase activity of the two major valves in the bird's respiratory system poses an interesting problem of respiratory motor coordination with respect to the differences in the laryngeal system of mammals.

It may, therefore, also provide some insight into the evolution of the syrinx.

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