

FUNCTIONAL SYRINGEAL ANATOMY OF THE
MALLARD. I. *IN SITU* ELECTROMYOGRAMS
DURING ESB ELICITED CALLING

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STUDENTS of phylogeny have realized that diversity in syringeal anatomy (particularly among the Anatidae) is an important taxonomic tool (Garrod 1875; Johnsgard 1961, 1971). Recently functional models of vocalization have appeared that attempt to relate sound production to anatomical structures (Gross 1964, Greenewalt 1968, Stein 1968, Gaunt et al. 1973, Gaunt and Wells 1973). To date the most detailed studies of syringeal anatomy concern passerine species (Miskimen 1951, Chamberlain et al. 1968, Ames 1971). Still for making physiological models of vocalization, nonpasserines offer several distinct advantages. They generally have relatively small repertoires of repetitive calls, have only two or three pairs of muscles operative on the syrinx and are large enough to facilitate *in situ* physiological measurements. Recently Youngren et al. (1975) took advantage of these features to investigate the neuromuscular control of vocalization in chickens. The considerable differences in the syringeal anatomy of Mallards (*Anas platyrhynchos*) suggested comparing neuromuscular control of vocalization in the two species to test the generality of their conclusions. The differences in syringeal structure between chickens and ducks include: relative size and skeletal attachment of syringeal-tracheal musculature, use of external vs. internal tympaniform membranes for sound production, and the enlarged osseous bulla of Mallard males.

The calls of Mallard ducklings are strident, slowly modulated, relatively pure tones with little or no harmonic complexity. In adults the vocalizations have such great harmonic complexity that the calls resemble human laryngeal sounds (highly pulsed, rippling membrane vibrations of Greenewalt 1968). Based on histological studies, Warner (1971) reported that the internal tympaniform membranes (I.T.M.) of adult male Mallards were 20 times as thick as those of adult females (0.750 vs. 0.04 mm). The voices of male and female ducklings are identical before age 41–45 days (Lockner MS), but the development of the anatomical differences has not been investigated (Abs (1969) considered only tracheal length). The present paper describes the neuromuscular control of sound production in adults and also the maturational changes in membrane thickness in ducklings.

METHODS

We bought 47 Mallards (35 ducklings, 6 adult males, and 6 adult females) from Wild Wings Game Farm, Hugo, Minnesota. Anatomical descriptions are based on 10

fresh adult syringes and 35 duckling syringes from 1 h to 57 days of age. The ducklings were frozen whole and then thawed and dissected. All dissections were made with the aid of a dissecting microscope (from 8 \times –40 \times). All syringeal muscles were traced to their point of origin on skeletal structures. After dissection all syringes were preserved in 70% ethyl alcohol and reexamined. Connective tissue and fat were removed and the innervation of all muscles was determined by tracing the entry of cranial nerves to the trachea and following branches to all muscles.

For histological studies, 35 μ frozen sections, embedded in albumen, were stained with cresyl violet. Syringes of older birds were first decalcified with Schmidt's fluid (10% EDTA in 4% formalin with 1% sodium acetate).

Physiological experiments were performed on 5 adult males, 7 adult females and 1 juvenile male, all of which were maintained under surgical anesthesia by periodic injections of sodium pentobarbital (Diabotal, 60 mg/cc, given at approximately 0.05 cc/h). Access to the tracheal muscles was gained by incising the skin from the clavicular junction to the top of the trachea, and rupturing the membrane of the interclavicular air sac (ICAS). Wick electrodes were used to record electromyograms (EMG's) from the tracheal muscles *in situ*, either by draping the wick over the muscle, or by looping it around the muscle to secure it during vocal activity. Vocalizations were elicited by electrical stimulation of the brain (ESB) delivered through a bipolar stimulating electrode to the low threshold midbrain calling area (for details of location and stimulus parameters, see Phillips and Youngren 1973). With this technique, we could elicit slow rahb and r b-r b calls from males and persistent quacks from females. One of us (F.R.L.) has worked with Mallard vocalizations for several years and the ESB elicited calls sound and appear graphically on spectrograms like calls given in free ranging birds. To obtain EMG's from respiratory muscles, fine wire (0.13 mm diameter, enamel insulated) electrodes were used. A small hook formed at the tip of the wire was inserted into the muscle with a 26-gauge hypodermic needle. The wire had a 0.25 mm bare tip as its active surface. EMG activity was led to Grass P5 or Tektronix type 122 preamplifiers and monitored on a Tektronix dual beam storage oscilloscope and a Grass audio monitor. EMG data were tape-recorded on a Tanberg-Sangamo Instrumentation recorder and permanent records were obtained by filming taped data from a CRO with a Grass 35 mm kymograph camera. Reversible blocks of the hypoglossal nerve were made by applying ice to the nerve.

RESULTS

GROSS ANATOMY

Tracheal structure.—The trachea consists of a variable number (114–134) of complete cartilaginous rings. When the trachea is fully compressed, the rings present a rhomboidal pattern in both ducklings and adults. Each ring is so structured that upon tracheal contraction the lateral portions of alternate rings on one side slide over the intervening rings, whereas on the contralateral side the pattern is reversed, i.e. the rings that slide under on one side, slide over on the other. The bronchial rings are smaller than the tracheal rings and are incomplete medially.

The first tracheal ring (counting from rostral to caudal) is immediately caudal to the ventral cartilaginous nodule of the urohyal in both males

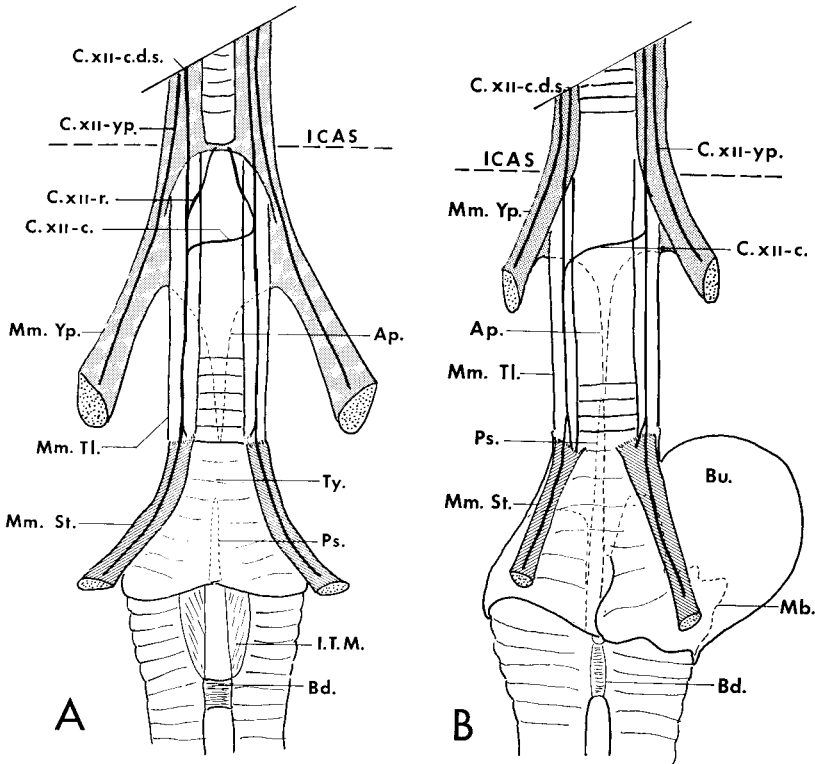


Fig. 1. Syringes of A. adult female Mallard, B. adult male Mallard, both ventral view. Relative size of nerves not drawn to scale: Ap., dorsal aponeurosis of Mm. ypsilotrachealis; Bu., osseous bulla; Bd., bronchidesmus; C. xii-c.d.s., cervicalis descendens superior branch of hypoglossal nerve; C. xii-c., crossover branch of hypoglossal; C. xii-r., recurrent branch of c.d.s. to "sling" of Mm. ypsilotrachealis; C. xii-yp., branch of c.d.s. to Mm. ypsilotrachealis; ICAS, boundary of interclavicular air sac; I.T.M., internal tympaniform membrane; Mb., membranous extension of bronchi within bulla; Mm. St., Mm. sternotrachealis; Mm. Tl., Mm. tracheolateralis; Mm. Yp., Mm. ypsilotrachealis; Ps., pessulus; Ty., tympanum.

and females. This nodule is covered with a fascial sheath that extends caudally onto the trachea.

Tracheal-bronchial junction.—Females: The syrinx, the organ of sound production, is composed of modified tracheal and bronchial rings. In females the anterior portion of the syrinx is an expanded bell-shaped syringeal drum composed of eight fused tracheal rings (Fig. 1A). The sound producing I.T.M.'s are in the medial wall of each bronchus (Gottlieb and Vanderbergh 1968). Inside the syringeal drum is the pessulus, a

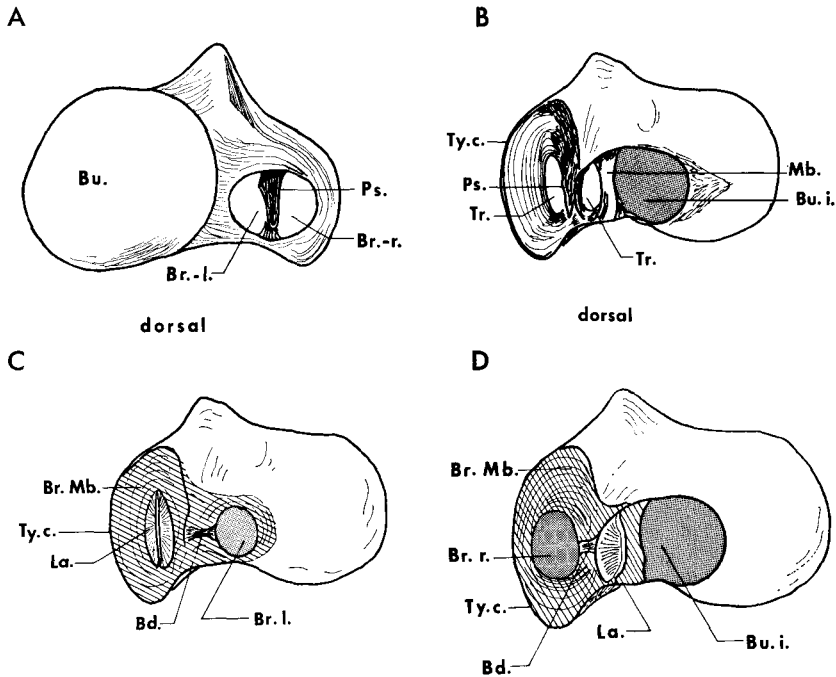


Fig. 2. Bulla of male Mallard. A, tracheal view. B, bronchial view. C, bronchial view with syringe moved caudally onto bronchi showing closing of right labial folds (Br. Mb.). D, bronchial view with syringe moved caudally onto bronchi showing closing of left labial folds (Br. Mb.); bulla has been transected. Bd., bronchidesmus; Br.-l., left bronchus; Br. Mb., bronchial membrane; Br.-r., right bronchus; Bu., osseous bulla; Bu. i., inside of bulla; La., labia of bronchial membrane; Mb., membranous wall of bulla; Ps., pessulus; Ty. c., caudal margin of tympanum.

vertical ossification extending to the fifth (counting caudal from the tracheo-syringeal margin) fused elements of the drum, both ventrally and dorsally. A sheet of connective tissue, the bronchidesmus, joins the two bronchi at the caudal margin of the I.T.M.

Males: The syringeal drum is composed of eight fused cartilaginous rings. As in most male Anatinae, there is an enlarged osseous bulla on the left side (Figs. 1B, 2A, 2B). The area between the bronchidesmus and the caudal margin of the bulla is filled with a thick layer of fat and connective tissue, leaving only a small (1-2 mm) potential space between the medial bronchial walls. The gross structure of the I.T.M. is clearly different from that in the female. The I.T.M.'s are thickened and even when the overlying fat and connective tissue is dissected away they are almost opaque, whereas the membranes of the female are quite thin and translucent. On the inner medial bronchial walls longitudinal folds run

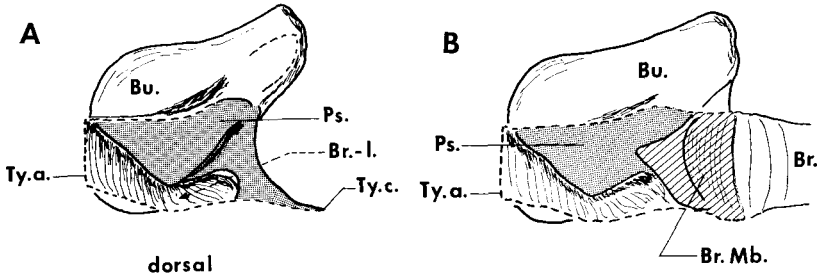


Fig. 3. Syrinx of male Mallard. A, lateral view with left side of tympanum sectioned (dotted lines) to show pessulus. B, lateral view with left side of tympanum sectioned (dotted lines). Syrinx moved caudally onto bronchi. Right labia (Br. Mb.) move against the pessulus occluding air flow through the right bronchus. Br., right bronchus; Br.-l., air pathway from left bronchus; Br. Mb., membrane of right bronchus; Bu., osseous bulla; Ps., pessulus (stippled); Ty. a., anterior margin of tympanum; Ty. c., caudal margin of tympanum.

rostrally to the bronchial-bulla junction. These appear to be derived from the slightly overlapping junctions of the medially incomplete bronchial rings. The inner bronchial walls are modified into membranous labial folds at the point where the bronchi meet the syringeal drum (Fig. 1B). We are not suggesting that these labial folds are homologous to the external and internal labia of the oscine syrinx (which are supported by rotating bronchial elements and presumably used as modulating oscillators). As the bulla moves caudally toward the bronchi these labia oppose one another and can close the bronchial lumen (Figs. 2C, 2D, 3B). The pessulus divides the syringeal drum as in the female (Fig. 3A). Air flows directly through the right bronchus, whereas it can take either of two pathways through the left bronchus: a ventral route straight through the syringeal drum or a dorsal spiral route through the bulla to the trachea (Fig. 4A, 4B).

Musculature.—Three paired extrinsic (originating and inserting on the trachea and/or bronchi rather than the syrinx itself as opposed to intrinsic muscles that originate and insert on the syrinx) muscles control the syrinx in Mallards, *Mm. tracheolateralis*, *Mm. sternotrachealis*, and *Mm. ypsilotrachealis* (Figs. 1A, 1B). In both sexes *M. tracheolateralis* arises on the first or second tracheal ring (counting caudally from the head). Each muscle descends along the lateral side of the trachea and inserts on the syringeal drum (usually the first, second, and third fused tracheal rings). The remaining two muscles are different in females and males, and are described separately for the sexes.

Females: The *M. ypsilotrachealis* is the largest of the three syringeal

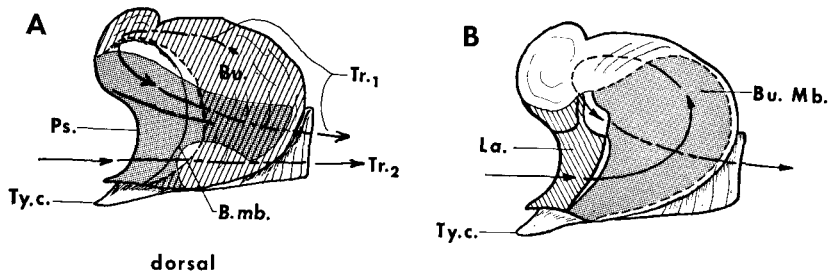


Fig. 4. Air flow patterns through osseous bulla of male Mallard. A, left lateral view showing two air pathways through bulla. Portion of bulla transected (indicated by dashed lines). In this position part of the air flow from the left bronchus proceeds directly into the trachea (pathway Tr.₂), and a portion of the flow proceeds in an upward spiral direction through the bulla and then into the trachea (pathway Tr.₁). B, left lateral view when bulla is moved caudally onto the bronchi. Left side of bulla transected (indicated by dashed lines). In this position the labium occludes the direct pathway into the trachea and the entire air flow from the left bronchus is routed in an upward spiral direction through the bulla (same pathway as Tr.₁ in Fig. 4A). B. mb., membranous inner wall of bulla; Bu., osseous bulla; La., labium of left bronchus; Ps., pessulus; Ty. c., caudal margin of tympanum.

muscles. In six females (age 1 h to 19 days) these muscles originated on the furcula. In larger birds, these muscles originate via a wide aponeurosis from the fascial sheath of *M. coracobrachialis*. In two birds (ages 12 and 17 days) the muscles originated from both the furcula and the *M. coracobrachialis*. The muscles run obliquely in an anterior-medial direction wholly within the interclavicular air sac (ICAS) and reach the trachea at its junction with the ICAS membrane. After reaching the trachea, each muscle runs anteriorly over the *M. tracheolateralis*. The fibers of *M. ypsilotrachealis* can be separated ventromedially, but not ventrolaterally, from those of the *M. tracheolateralis*. The fibers run cephalad to the point of emergence of a branch of C. xii (hypoglossal) at approximately the anterior one-third of the trachea. Anterior to this point the fibers of the two muscles are not separated by areolar connective tissue and are indistinguishable on gross dissection. The *M. ypsilotrachealis* has been described as arising from the clavicle and inserting on the trachea with the belly of the muscle intimately associated with the skin of the neck (George and Berger 1966). *M. ypsilotrachealis* of the female Mallard is significantly different because of its origin deep within the thoracic cavity and lack of adherence to the dermis of the neck.

In adult females and older ducklings (past 17 days in this study) the two *M. ypsilotrachealis* are connected ventrally at the point where they join the trachea (at the ICAS margin). In the youngest birds this con-

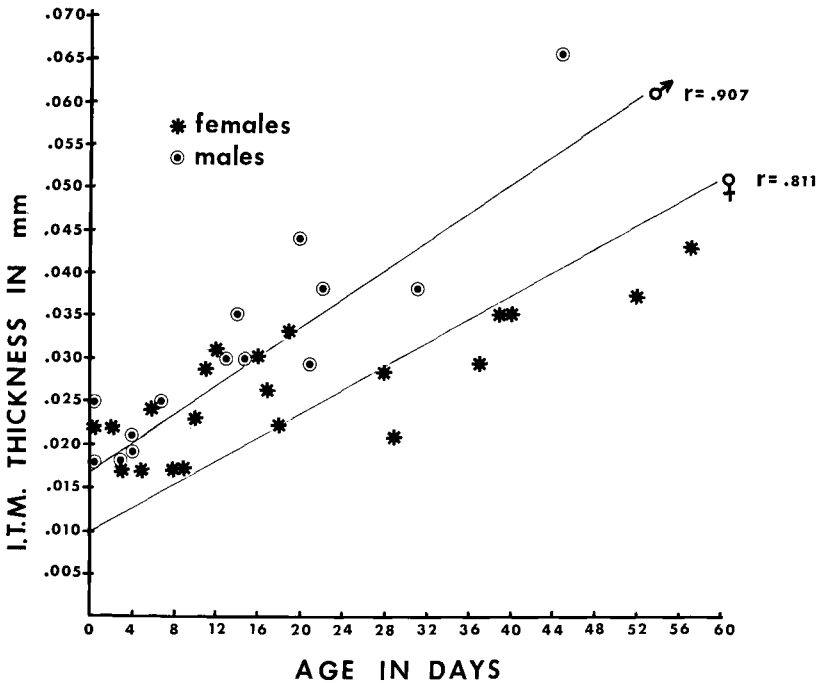


Fig. 5. Thickness of internal tympaniform membranes of Mallard ducklings (age 1 h-57 days). Regression lines and correlation coefficients for males and females.

nection contains only a few muscle fibers, but it is much larger in the older ducklings and adults. Dorsally, most fibers turn cephalad to course up the trachea, but a few fibers insert on a large dorsal aponeurosis that extends from the caudal margin of the syringeal drum (over the pessulus) and runs anteriorly. Thus the ventral and dorsal fibers of the *M. ypsilotrachealis* form a sling that encircles the trachea at the point where the ICAS membrane joins the trachea (Fig. 1A).

The *M. sternotrachealis* is smaller than the *M. ypsilotrachealis* and originates from the coricoidial-sternal process of the sternum (costal process of McLeod et al. 1964; the craniolateral process of Koch 1973) in all females examined. *M. sternotrachealis* passes ventral to the subclavian arteries and inserts on the syringeal drum (within the ICAS). Laterally, the fibers insert over those of the *M. tracheolateralis*, medially they insert on the first four fused tracheal rings of the syringeal drum (counting caudally from the syringeal-tracheal junction).

Males: In males the *M. ypsilotrachealis* is much smaller than in females. In most males the pair originate within the ICAS membrane at its ventral

apex near the sternum. The two Mm. ypsilotrachealis may have a common origin from the furcula and then divide to run in the membrane. They are exceedingly thin, although wide (ca. 2 mm in adults). The origin is quite variable; in five males (ages 10 h to 15 days) the origin was wholly from the furcula much as in females. In two males (ages 3 and 14 days) one of the muscle pair originated from the furcula, the other in the membrane. In all older males the origin was wholly within the ICAS membrane. At the trachea the fibers divide, one group passes cephalad up the trachea, the other group runs over the trachea to insert on the fascia between the trachea and esophagus and meet ventrally at the ICAS membrane forming a small sling around the trachea as in the females.

The Mm. sternotrachealis are larger than the Mm. ypsilotrachealis and, in adults, those of the males are larger than the females'. The origin is identical to that in females (coracoidal-sternal process of the sternum). The insertion is on the syringeal drum just cephalad to the bulla. In adults, the left and right M. sternotrachealis may insert differently. The fibers of the left M. sternotrachealis cross the osseous bulla without inserting on it, and insert on the third and fourth (counting caudally from the syringeal-tracheal junction) tracheal rings of the syringeal drum. Small slips also invest the junction of the medial surface of the bulla and the lateral surface of the trachea. The right M. sternotrachealis crosses the osseous bulla and begins insertion on the fourth fused cartilaginous ring and completes insertion on the first (counting caudally from the syringeal-tracheal junction). A small slip may also insert on the first moveable tracheal ring. In some males, fibers begin to diverge from the right M. sternotrachealis at the fifth fused ring of the syringeal drum and at the second fused ring, these have formed a separate head. When present, the fibers of this head do not insert on the bulla but cross the trachea and insert on the left tracheal surface of the left M. tracheolateralis and interdigitate with its fibers.

Innervation.—Cranial nerve xii, the hypoglossals, innervate all three pairs of syringeal-tracheal muscles (Figs. 1A, 1B). The nerves exit from the cranium at approximately the level of the 10th tracheal ring (counting caudally from the head) and join the trachea some 8 to 15 tracheal rings caudally. Both the point at which the two nerves join the trachea and also the number of definitive branches that enter the M. tracheolateralis vary greatly. At the least, each hypoglossal nerve sends one branch up the M. tracheolateralis to its origin, and a descending branch, the cervicalis descendens superior (c.d.s.), enters the fibers of the M. tracheolateralis. Although the descending branch is clearly visible to the unaided eye, it runs within the fibers of the M. tracheolateralis rather than on the surface. When more than one descending branch is present

(there can be as many as three) they join some 6 to 12 tracheal rings caudal to their point of entry. Approximately one-third of the way down the trachea each hypoglossal gives off a smaller branch to the *M. ypsilotrachealis*. These branches continue to the origin of each *M. ypsilotrachealis* without further obvious branching.

At the syringeal drum each hypoglossal nerve continues into *M. sternotrachealis*, after giving off small branches to the insertion of the *M. tracheolateralis*. The larger branches to *M. sternotrachealis* immediately turn deep into the muscle where they give off small collateral branches. There is no separate branch of the hypoglossal nerve to the second "crossover head" of the right *M. sternotrachealis* when present in males.

Inside the ICAS, the left hypoglossal nerve often sends a crossover branch to the right hypoglossal. This branch is always on the ventral surface of the trachea and joins the right hypoglossal some one to four tracheal rings below its origin on the left. The presence of the crossover is variable. It occurred in all adults examined although it was much smaller in the females. We could not find a crossover branch in seven of the ducklings, although this does not seem to be an age function (it was present in a 3-day-old female and 4-day-old male).

In the females recurrent branches of *C. xii* innervate the sling portion of the *M. ypsilotrachealis*. Just anterior to the left-right hypoglossal crossover each hypoglossal gives off a very small ascending branch that innervates the ventral connecting fibers of the *M. ypsilotrachealis* (Fig. 1A).

Histological studies.—The internal tympaniform membranes of the 35 ducklings sampled were progressively thicker with age (from 0.017 mm for the youngest ducklings to 0.043 mm in a 57-day-old female and 0.071 mm in a 45-day-old male, Fig. 5). Sexual differentiation of the membranous structures of the syrinx are evident at age 21 days. In females, the I.T.M.'s are of almost uniform thickness from the pessulus to their caudal apex at the bronchi whereas in older males (31 and 45 days) the caudal margins of the I.T.M.'s are thickened and folded, particularly in the right bronchus. Additionally, a large membranous fold that lines the bulla appears at age 31 days. It thus appears to us that the labia develop from the caudal portion of the I.T.M., although additional work on ducklings past age 45 days is needed to confirm this.

PHYSIOLOGICAL STUDIES

Muscular activity.—*Mm. tracheolateralis*: Only the *Mm. tracheolateralis* contract during silent respiration under general anesthesia (Fig. 6A). When referenced to *M. obliquus abdominus externus*, an expiratory muscle, neither the *Mm. ypsilotrachealis* nor *Mm. sternotrachealis* are active during respiration. We were able to record only weak EMG's from *Mm.*

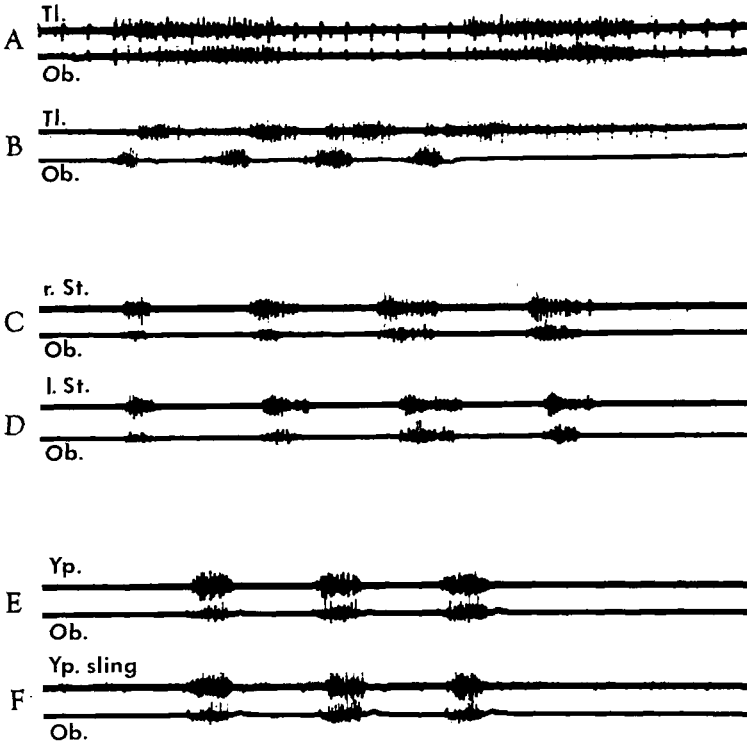


Fig. 6. A, nonvocal respiratory EMG activity of *M. tracheolateralis* (TI.) in an adult female Mallard, referenced to the activity in the expiratory muscle *M. obliquus abdominus externus* (Ob.). Time scale, 50 mm/sec (smaller regular spikes represent ECG). B, EMG activity of *M. tracheolateralis* during ESB-induced vocalization in an adult female Mallard. Time scale, 100 mm/sec. C, D, EMG activity of the right (r. St.) and left (l. St.) *M. sternotrachealis* of an adult male Mallard during ESB-induced vocalization. Time scale, 100 mm/sec. E, EMG activity recorded during ESB-induced vocalization from *Mm. ypsilotrachealis* and *M. obliquus abdominus externus*. Time scale, 100 mm/sec. F, EMG activity recorded during ESB-induced vocalization from the ventral center of the tracheal sling formed by the *Mm. ypsilotrachealis* in an adult female Mallard. Time scale, 100 mm/sec.

tracheolateralis cephalad to the ICAS where they are covered by *Mm. ypsilotrachealis*, as *Mm. ypsilotrachealis* are silent during respiration. During ESB evoked vocalizations, EMG activity in the *Mm. tracheolateralis* follows that in the *M. obliquus abdominus externus* (Fig. 6B).

Mm. ypsilotrachealis: These muscles contract only during calling and then in phase with *M. obliquus abdominus externus* (Fig. 6E) and EMG activity lags behind that of the first expiratory activity by 10–30 msec.

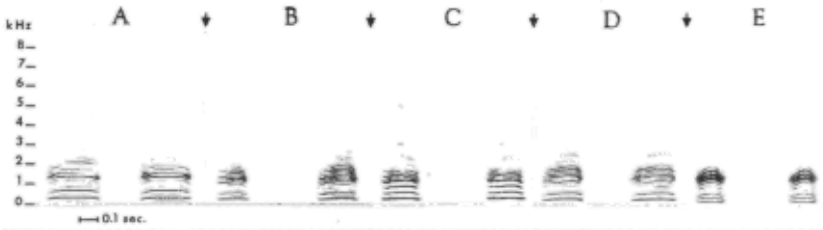


Fig. 7. Midbrain ESB evoked "quack" calls from an adult female Mallard. All calls were evoked from the same site with the same stimulus parameters under general anesthesia. A, ICAS intact. B, left bronchus clamped shut, skin over sac held closed. C, right bronchus clamped shut, sac held closed. D, both bronchi open, sac held closed. E, bilateral transection of C. xii (hypoglossal).

When stimulus trains are used to evoke three or more repetitive calls the largest latency is always on the first burst. By the third call in the series the EMG activity of *M. ypsilotrachealis* coincides with or precedes that of the expiratory muscles. The fibers of the ventral crossover connecting the two *M. ypsilotrachealis* at the level of the ICAS membrane burst synchronously with those of the main muscle mass (Fig. 6F).

Mm. sternotrachealis: Like the *Mm. ypsilotrachealis*, the *Mm. sternotrachealis* are active only during vocalization (Fig. 6C, 6D). Their activity only slightly lags behind that of the expiratory muscle (approximately 10 msec). In fact, occasionally when using stimulus trains in males, the first burst of a series was the *Mm. sternotrachealis* alone. All succeeding bursts were accompanied by expiratory activity as well; i.e. the first muscular activity after the stimulus onset was a contraction of the *Mm. sternotrachealis* after which the *Mm. sternotrachealis* and *M. obliquus abdominus externus* contracted in phase. This suggests that the call generator mechanism has differential access to *Mm. sternotrachealis* and the expiratory muscles (cf. Phillips and Peek 1975). Simultaneous recordings from the left and right *Mm. sternotrachealis* show that they contract synchronously.

Innervation.—All three pairs of syringeal-tracheal muscles are innervated by the hypoglossal nerve (C. xii). In dissection we could find no branches of the vagus nerve (C. x) to any of these muscles, although small branches to the esophagus and crop were easily visible. When the vagus was stimulated (0.5 msec duration, 100 pulses/sec, 10 volts) we got marked bradycardia (from EKG electrodes) but could not detect any EMG activity in any of the tracheal-syringeal muscles.

Mm. tracheolateralis: Ipsilateral hypoglossal cold block of either the left or right *M. tracheolateralis* immediately eliminates all EMG activity

whereas a contralateral block has no effect. Ipsilateral blockage was complete both during nonvocal respiration and ESB evoked calling.

Mm. ypsilotrachealis: All EMG activity during ESB evoked calling from either the right or left *M. ypsilotrachealis* ceases with ipsilateral hypoglossal cold block. During calling normal EMG activity returns with the next expiratory burst after the ice is removed. Contralateral cold block of either the left or right hypoglossal nerve has no effect. These results confirm our anatomical descriptions of the innervation of these muscles, i.e. each is innervated by a branch from the hypoglossal nerve approximately one-third of the distance from the larynx to the ICAS membrane.

Mm. sternotrachealis: Ipsilateral hypoglossal cold block does not eliminate EMG activity from either the left or right *M. sternotrachealis*. In females ipsilateral blocks of the right *M. sternotrachealis* reduce the EMG response slightly. Contralateral C. xii block of the right *M. sternotrachealis* also reduces EMG response, but simultaneous block of both hypoglossals is the only way to eliminate all EMG activity in it. In males, only bilateral C. xii block eliminated the EMG from the *M. sternotrachealis*. Thus, the *Mm. sternotrachealis* but not the *Mm. ypsilotrachealis* nor *Mm. tracheolateralis* receive innervation via the left-right crossover branch of the hypoglossal nerve. The fact that we could not effect a complete ipsilateral block of the left *M. sternotrachealis* means that either our blocking technique was not effectively reaching all nerve fibers or that a small twig from the right hypoglossal crosses to the left hypoglossal.

Patterns of air flow.—In anesthetized adult females, all vocalizations cease when the ICAS is opened completely to the atmosphere. Stimulus trains in these preparations produce repetitive bursts of the syringeal-tracheal musculature and expiratory muscles, but much of the air flow escapes through the open sac (cf. Gaunt et al. 1973, Gaunt and Wells 1973). When the ICAS is open in chickens, occlusion of the secondary bronchi that lead into the ICAS eliminates the alternate air pathway and restores the tracheal flow, yet the birds are still mute (Youngren et al. 1975), thus demonstrating that pressure in the ICAS is necessary for call production. Holding the skin closed over the ruptured sac restores vocalizations in adult females (Fig. 7A, 7D). Anatomically the two sides of the adult female syrinx are identical. In order to determine the role of each I.T.M. in call production, we alternately clamped the left bronchus and right bronchus with an artery clamp in females implanted with mid-brain call site electrodes. Clamping either bronchus (when the skin over the sac was held shut) reduced the intensity of the vocalizations. Thus,

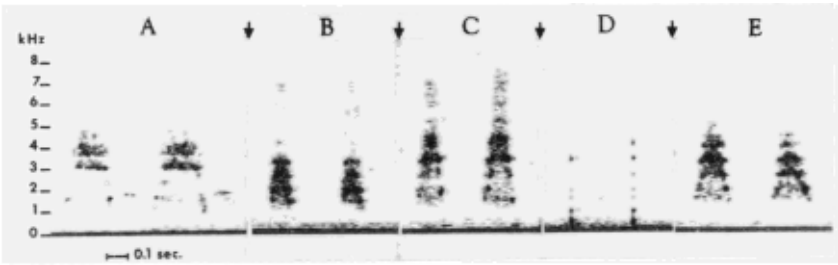


Fig. 8. Midbrain ESB evoked rãb calls from an adult male Mallard. All calls were evoked from the same site with the same stimulus parameters under general anesthesia. A, ICAS intact. B, ICAS completely open to the atmosphere. C, right bronchus clamped shut, skin over sac held closed. D, left bronchus clamped shut, sac held closed. E, both bronchi open, sac held closed.

adult females can call when air passes over either the right or left I.T.M. (Fig. 7B, 7C, 7D).

Adult male Mallards could still vocalize, although at reduced intensities, when the sac was completely ruptured (Fig. 8A, 8B). In one male with a weak call site, we had to occlude the secondary bronchi to reinstate calling, but in all males we could evoke calling with no positive ICAS pressure. These results are consistent with previous studies on ICAS pressure and vocalizations in unrestrained birds (Lockner and Murrish 1975) and are expected in view of the fact that the males do not possess the thin internal tympaniform membranes.

We constructed a pressure chamber to study excised syringes and found that external pressure was not necessary for sound production in drake syringes. We could produce definite rãb calls by blowing through the bronchi. When the tube to the left bronchus was clamped off, we could not produce calls. We tested these findings *in situ*, by alternately clamping the right and left bronchi in males with electrodes in the midbrain call site, and holding the skin closed over the ICAS when stimulating the birds. In males the rãb call could be elicited when the right bronchus was clamped (Fig. 8C, 8D, 8E). Closing the left bronchus eliminated all vocalizations. When the left bronchus was clamped shut we could hear air passing through the larynx and bill. Increasing the current or pulse duration had no effect. Thus, the rãb call is produced when air passes through the osseous bulla.

As the bulla is present at hatching (begins development at 14th embryonic day), but male ducklings have thin translucent internal tympaniform membranes and no opposable labia, we repeated the clamping experiment on a 30-day-old male duckling. We implanted electrodes

in a midbrain call site that elicited distress peeping. Regardless of which bronchus was clamped, this duckling could vocalize, i.e. distress calls are produced when air passes through either bronchus.

DISCUSSION

All three pairs of syringeal-tracheal muscles are innervated by the hypoglossal nerve (C. xii). That is not to say that vagal (C. x) innervation does not exist, but our results on Mallards and chickens (Youngren et al. 1975) indicate that any vagal innervation would have to be afferent. Only the Mm. sternotrachealis receive bilateral hypoglossal innervation. Thus the right M. sternotrachealis is partially innervated by a branch of the left hypoglossal. Nottebohm (1971) referred to this phenomenon as "neural lateralization" and discussed it in terms of handedness and cerebral dominance. Our studies on Mallards and chickens suggest that neural asymmetry is a more generalized phenomenon and may have no special significance in call production by these two species.

Greenewalt's (1968) model of vocalization involves three components; air flow, positive ICAS pressure, and tension of syringeal musculature. The resultant of these three factors determines the vibratory mode of the tympaniform membranes and thus sound production (Gross 1964, Gottlieb and Vandenberg 1968). In Mallards the general function of the three pairs of syringeal-tracheal muscles can be hypothesized as follows:

Mm. tracheolateralis contracts during expiration even when the birds are silent. Contraction of these muscles pulls the syrinx cephalad, which would tense the I.T.M.'s in females and ducklings, effectively preventing them from vibrating in the bronchial air stream and so prevent calling during expiration. During calling, contraction of Mm. ypsilotrachealis and Mm. sternotrachealis antagonize Mm. tracheolateralis, pulling the syrinx caudad onto the bronchus. In females this would relax the tension in the I.T.M.'s allowing their excursion into the bronchial air stream where they would be forced into vibration.

In the absence of these antagonistic forces (e.g. bilateral hypoglossal denervation) females can still call, as the air flow moves past the internal tympaniform membranes when the ICAS is at superatmospheric pressure (Fig. 7E). The action of Mm. ypsilotrachealis is also antagonistic to Mm. tracheolateralis. However, in Mallards the two muscles are connected ventrally at the ICAS membrane and also insert on the dorsal aponeurosis, forming a sling around the trachea. During calling this sling contracts phasically with the bodies of the Mm. ypsilotrachealis and may function to stabilize the trachea where it passes through the ICAS membrane.

Although *M. ypsilotrachealis* is strikingly sexually dimorphic in both size and point of origin, we cannot, at this point, ascribe any functional significance to this phenomenon.

Although no *in situ* physiological data exist for vocalization in passerines, Ames (1971: 134) wrote "it seems to me that the syringeal muscles, if they increase membrane tension, would tend to draw the internal tympaniform membranes out of the bronchus, reducing constriction of the passage." However, *Mm. sternotrachealis* are not intrinsic syringeal muscles, i.e. they do not originate and insert on the syrinx, and in Mallards and chickens clearly could not increase membrane tension. This suggests that their primary function may be to reduce tension on the internal tympaniform membranes rather than one of active modulation. The frequency modulations typical of the calls of Mallard ducklings (Lockner MS) and chicks may be passive phenomena. Bilateral section of the hypoglossal nerves in chicks neither mutes the birds nor eliminates the frequency modulation of the calls (Phillips and Peek 1975). Andrew's (1973) chicks could still modulate the beginning and end of their calls after section of extrinsic syringeal musculature. Nottebohm (1971) reported that bilateral hypoglossal denervation rendered adult chaffinches "virtually aphonic" and they produced wheezing sounds on inspiration. His spectrograms show these sounds are obviously modulated, and the modulations must be passively produced as the syrinx was denervated. Bilateral hypoglossal denervation does not appreciably change the calls in adult female Mallards (Fig. 7E).

In adult male Mallards *Mm. sternotrachealis* pull the bulla caudally onto the bronchi. We suggest that in producing the r**ä**b call, the membranous labial folds inside the right side of the tympanum oppose one another, thus directing air flow through the bulla, allowing the labial folds to vibrate in a manner similar to the mammalian vocal cords. The bulla also contains labial folds that can selectively direct the gas flow into the trachea or shunt the flow in a spiral direction through the bulla. In either case these folds cannot oppose one another if the syrinx is pulled cephalad from the bronchi. Although this valving mechanism can be duplicated in excised syringes, we have not as yet been able to demonstrate the functional mechanisms *in situ*.

The spectrographic structure of the male call is, as Greenewalt (1968) pointed out, reminiscent of human laryngeal sounds. He attributes this to complex rippling vibration of the I.T.M. In fact the extreme thickness of the I.T.M. in males (0.750 mm, Warner 1971), the deposition of fat and connective tissue covering the bronchi, the presence of opposable labia, and the fact that adult males can call with no ICAS pressure

suggest to us that rāb calls are not produced by I.T.M. vibrations, but by movement of air past the labia.

Developmental changes are obvious in the ducklings. The I.T.M.'s are clearly present in young males, thus anatomically male and female ducklings are similar (except for the bulla). The dominant call frequency decreases steadily from approximately 3200 Hz at hatching to approximately 1700 Hz at age 41 days (Lockner MS). Relative (our specimens had been frozen) I.T.M. thickness increased progressively from hatching to age 57 days in the 35 duckling syringes we examined. Even though thinner membranes produce higher frequency sounds, the relationship between I.T.M. thickness and call frequency is probably not linear. Factors such as membrane area and bronchial lumen cross section will have to be considered to formulate a realistic model.

As the sexual divergence in calls does not occur until age 41–45 days, the developmental changes need to be studied further in specimens beyond 40 days of age. Syringeal labia in males are not present in ducklings of 14 and 15 days but are always present after 20 days of age, although at this stage the labia cannot oppose one another to occlude the bronchial air flow. In the 45-day-old male, labia were present but the I.T.M.'s were also present and freely moveable. Abs (1969) has argued that the "stimmbruch" or break in the voice that occurs at age 27–41 days is produced by tracheal resonance. It seems to us that the increases in I.T.M. thickness coupled with the fact that reductions in ICAS pressure in young (approximately 10-day) ducklings result in a striking increase in the harmonic complexity of the calls (Lockner and Murrish 1974) argue that maturational changes in the syrinx are critical in determining the observed maturational changes in call development (Abs 1969, Andrew 1973, Lockner MS).

Even though we studied all three syringeal-tracheal muscles *in situ*, we cannot ascribe specific functions (other than turning sound on or off) to any of these muscles in terms of their actions in producing specific calls. They obviously interact with both the volume and temporal properties of the bronchial gas flow to effect the diversity in call intensity and temporal patterns. The syringeal anatomies of Mallards and chickens show significant differences: placement and shape of the pessulus, size and insertion of Mm. ypsilotrachealis, use of internal rather than external tympaniform membranes, and the presence of the osseous bulla and labial folds in male Mallards. Yet the patterns of muscular activity are essentially similar with the exception of the sling component of the Mm. ypsilotrachealis in Mallards. In both species Mm. tracheolateralis contract during silent respiration and the Mm. sternotrachealis and Mm. ypsilotrachealis are active only during vocalization.

As the vocal system is actually part of the respiratory system and both must use the same respiratory muscles to effect air movement, vocal diversity in terms of species and individual differences could be most easily produced by changes in peripheral rather than in central mechanisms and structures. Based on a study of passerine syringeal anatomy, Ames (1971: 136) concluded "The major factor in vocal diversification in the oscines has been changes in the nervous system, rather than in syringeal structure," which may be true for some of the subtle differences in song across oscine species. Obviously chickens and ducks produce different calls, yet the neural controls of the syrinx are similar in the two. In fact the central nuclei and pathways that terminate in the hypoglossal nuclei that have been implicated in sound production show striking similarities in fish, anurans, birds, and mammals (for review see Phillips and Peek 1975).

We believe the techniques reported here afford a unique opportunity for the study of the physiological control of behavior. The advantages in studying motor output systems have been argued several times. Vocalizations form a class of motor patterns that can be reliably elicited with ESB and are easily quantified (in contrast to simpler reflexes (Sherrington 1947) and more complex variable behavior (von Holst and St. Paul 1963)). The vocal system is relatively simple in terms of effectors and from this output one can work "upstream" to central mechanisms in the medulla, midbrain, and forebrain. It seems especially fruitful to consider how differences in peripheral structures (i.e. syringeal mechanisms) modify the output. For example in the Mallard, syringeal modifications (osseous bulla and membrane structure) radically change the output. To determine the exact functional relationship ultimately between syringeal-tracheal mechanisms and the respiratory pump it is essential that one be able to elicit the same vocalizations repeatedly under the same experimental conditions. ESB provides the necessary tool to do this. It must be pointed out that, although one can affect the intensity and temporal pattern of a given call by varying the stimulus parameters, it is not possible to elicit a continuum of calls from any given site. A function (e.g. modulation) other than on-off control of sound by the syringeal muscles remains to be determined.

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SUMMARY

Based on fresh dissections of 47 birds and *in situ* electromyograms of 12 adults, we have described the functional syringeal anatomy of the Mallard. In both sexes the three syringeal-tracheal muscles, Mm. tracheolateralis, Mm. sternotrachealis, and Mm. ypsilotrachealis, are all innervated by the hypoglossal nerve (C. xii). M. sternotrachealis is bilaterally innervated by a left to right crossover branch of the hypoglossal within the interclavicular air sac. Only the Mm. tracheolateralis contract during vocally silent respiration. During vocalization (evoked with electrical stimulation of midbrain calling sites) all three muscles are active and phasic with expiration (as referenced to EMG activity in the M. obliquus abdominus externus). The function of syringeal-tracheal musculature during vocalization is discussed in terms of proposed hypotheses of vocalization (cf. Greenewalt 1968).

In females and young ducklings, the sound producing internal tympaniform membranes (I.T.M.'s) are easily visible and translucent, and the birds can produce normal calls when either bronchus is clamped shut. The thickness of the I.T.M. increases progressively with age in both males and females (we examined 35 syringes of ducklings from 1 h to 57 days of age). In adult males the I.T.M.'s are extremely thickened and covered with connective tissue and fat. Both the right and left bronchi contain opposable membranous labia (within the tympanum) which can act as valves to direct the air flow through the osseous bulla. Based on experiments on excised syringes in a pressure chamber and *in situ* recordings from birds with a ruptured interclavicular air sac, we suggest that the rüb calls of adult males are not produced by the I.T.M.'s. The rüb call is only produced when air passes through the left bronchus and osseous bulla. The pathways of air flow through the bulla are described.

Although the sexual dimorphism in syringeal structures and vocalizations is marked, the neuromuscular activity in males and females is identical. Call diversity is discussed in terms of central and peripheral mechanisms.

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