EFFECTS OF DENERVATION OF THE TRACHEO-SYRINGEAL MUSCLES ON FREQUENCY CONTROL IN VOCALIZATIONS IN CHICKS

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ABSTRACT.—Audiospectrograms of chick twitter, peep, and trill vocalizations showed that denervation of the extrinsic syringeal muscles reduced frequency ranges and amplitudes of all three calls but that appreciable frequency modulation remained. Electromyograms from freely moving, awake chicks showed that the abominal (expiratory) muscles twitch and relax for each sound pulse of trills (up to 40–50/s). Comparison of these results with reports for songbirds supports the idea that the extrinsic syringeal and expiratory muscles play similar roles in both groups. *Received 10 June 1980, accepted 6 October 1980.*

ALTHOUGH anatomical and some physiological studies strongly suggest that the extrinsic syringeal muscles play an important role in the production and modification of avian vocalizations (Miskimen 1951, Brockway 1967, Peek 1972, Andrew 1973), several have failed to support such a role for them (Gross 1964, Smith 1977, Brackenbury 1978a). The persistence not only of sound production but also of appreciable frequency modulation after sectioning of the muscles raised the possibility that the correlations reported between vocalizations and EMG activity (Youngren et al. 1974, Gaunt and Gaunt 1977) were somehow spurious and that the muscles played no necessary role in vocal control. The present experiments were designed to test this hypothesis more thoroughly.

METHODS AND MATERIALS

Chicks.—Chicks (*Gallus gallus*) were hatched in our laboratory from a cross between Rhode Island Red cocks and Barred Rock hens. They were hatched and housed in groups except during surgery and recording.

Surgery.—All chicks were anesthetized with 1.8–2.4 mg of veterinary pentobarbital sodium (Diabutal, Diamond Laboratories, Inc.) injected into a leg muscle (gastrocnemius). Surgery was performed under a Zeiss operating microscope with coaxial light source.

Experimental groups.—(1) To test the effects of total denervation of the syrinx, the muscle and attached nerve (cervicalis decendans superior or c.d.s.) were removed bilaterally from 6–8 tracheal rings just caudal to the glottis in six chicks (Cut XII-High). In adults this procedure severed the only innervation of the two pairs of muscles (m. tracheolaterlis, m. sternotrachealis), eliminating their activity (Youngren et al. 1974). In all these experiments, we have assumed that these muscles have no innervation in chicks that is absent in adults: careful dissection has revealed none. In six sham-operated controls from the same hatch, the neck skin was incised and sutured as a control for anesthetic and trauma affects (Operated Control).

(2) To test for effects of denervation on m. sternotrachealis, the muscles and nerves were removed from the 6–8 tracheal rings immediately cephalad of the interclavicular air sac membrane (Cut XII-Low). This left most of m. tracheolateralis functional but, based on our anatomical studies of adults (Youngren et al. 1974), completely denervated m. sternotrachealis without interfering with the interclavicular air sac membrane or with any passive mechanical action of the muscle upon the syrinx. The six sham-operated chicks from (1) above (Operated Control) served as controls for this group also.

(3) To compare the effects of direct interference with m. sternotrachealis to those of denervating it, the interclavicular air sac was opened in six chicks, and the sternotrachealis muscles were severed bilaterally at their insertion on the trachea. Their free ends were allowed to drop into the sternal cavity (Cut Muscles). To control for possible effects of rupturing the interclavicular air sac membrane, the sacs were

opened to the same extent as in the experimental birds in six control chicks, but the sternotrachealis muscles were left intact (Rupture Air Sac). In both of these groups the neck skin was sutured tightly to prevent air leakage. A group of six intact chicks that were never anesthetized was tested as the others to provide normal calls for comparison (Intact Control).

(4) In six additional chicks a third pair of muscles, m. ypsilotrachealis, was sectioned and effects on vocalizations analyzed. In four of these, m. sternotrachealis and m. tracheolateralis were denervated as well (by sectioning c.d.s. high in the neck). In only two was m. ypsilotrachealis alone affected.

Eliciting and recording vocalizations.—Three types of calls that Andrew (1963) described and named were studied; peeps, twitters, and trills. These three types of calls include the entire range of frequency modulations that occur in a chick's vocal repertoire. Examples of each from intact chicks are displayed under the PRE headings of Fig. 1. Chicks were deprived of food for 3–5 h prior to recording, then put into an oval test pen 76 cm by 56 cm with 38-cm-high solid walls lined with 12 mm of foam. The floor was padded, and the whole pen was housed inside a sound isolation room (Industrial Acoustics Co., Inc.) that eliminated noise from other birds and activities in the bulding. The pen was lighted by an overhead, 100-W incandescent bulb. The recording microphone was hung 8 cm above the center of the pen floor. Calls were recorded at a tape speed of 19 cm/s (7½ ips) on a Sony TC 353 D tape deck, and the chicks were monitored either through a one-way mirror in the sound isolation room wall (peeps) or by the experimenter in the room itself (twitters and trills). Peeps were evoked simply by leaving the chick alone in the test pen. A few chicks that failed to peep with the room lights on did so quickly when the lights were turned off. Twitters were reliably evoked by talking softly to the chick and/or by placing a hand in the test cage near it. Hungry chicks nearly always responded to this by feeding and twittering. Trills were elicited by suddenly picking up a feeding or twittering chick by one leg or, better, by first getting the chick to feed, then pulling it sideways by one wing. This didn't disturb the chick enough to cause peeping unless the action persisted. Trills were difficult to evoke if the chicks were peeping or showed other signs of being frightened.

All three types of calls were recorded from each of the experimental and sham-operated control chicks on the second day after surgery. Comparable recordings were made from six intact controls.

Verification of nerve and muscle section.—Following testing the experimental chicks were killed and their tracheae examined under a dissection microscope.

Measurement of frequency modulations.—Samples of four to six calls from each chick from each test period were visualized with the audiospectrograph (Sona-Graph 6061B, Kay Elemetrics), and measurements of maximum and minimum frequency of the fundamental frequency and of its duration were made. Harmonics were not included in the measurements. Peeps were sampled just after peeping had reached its loudest as judged subjectively. Twitters were sampled just as the hungry chick discovered food, a time when the calls were loudest and most rapidly uttered. The loudest and longest trills from each chick were selected for analysis.

Treatment effects on each measure were evaluated with one-way analysis of variance with individual *t*-tests for all possible pairs where significance for the overall comparison exceeded 0.05. Primary attention was paid to comparisons between Operated Controls and the two Cut XII groups. In addition, a Fisher Exact Probability Test for equality of proportions in independence of treatment and call-not-call was used to test the probability that the number of birds that trilled in the Low-cut group was the same as that for controls (Operated Controls).

The chicks recovered from the anesthesia and surgery very quickly: they fed and drank as soon as they could stand, and by the next day they seemed completely normal. Only the data from the second day after surgery are analyzed here.

Electromyograms.—To determine whether the expiratory muscles contributed to the trills that persisted after muscle or nerve section, a pair of enamel-insulated nichrome wires 0.127 mm in diamter, with 1-mm bare tips bent into hooks, were implanted in the abdominal oblique muscle of each of two 14-day-old female chicks. When the chicks had recovered from anesthesia, EMG's were recorded on one channel of a Sony stereo tape recorder (after amplification by a Tektronix P-122 pre-amplifier), and calls were simultaneously recorded via a microphone connected to the other channel. Tape speed was 9.5 cm/s. The records were visualized by photographing the face of a dual-beam oscilloscope with a Grass oscillographic camera at a film speed of 100 mm/s.

RESULTS

Post mortem examinations showed that section of the appropriate nerves or muscles was complete in all experimental chicks.

Cutting the nerves (Cut XII-High) to the tracheal muscles (m. tracheolateralis,

TABLE 1. Characteristics of peep vocalizations in control and experimental groups of chicks.

	Intact Control	Operated Control	Cut XII High	Cut XII Low	Rupture Air Sac	Cut Muscles
Maximum frequency						
Mean	5,190^{a}	4,920^{ab}	4,010^{c}	2,970^{c}	4,420^{bc}	3,150^{d}
SE	200	130	130	70	190	110
Minimum frequency						
Mean	1,810^{a}	1,900^{a}	2,180^{a}	1,880^{a}	1,890^{a}	1,900^{a}
SE	50	50	120	100	120	50
Range (maximum–minimum)						
Mean	3,380^{a}	3,010^{a}	1,830^{c}	1,100^{d}	2,540^{b}	1,250^{d}
SE	220	160	100	50	170	100
Number of chicks peeping	6	6	6	5	6	6

[a] All six chicks of each group were tested for each measure in this and the following tables. All numbers in a horizontal row of this and subsequent tables that have different superscripts differ ($P < 0.05$). Those with the same superscript do not differ ($P > 0.05$).

m. sternotrachealis) reduced, but did not abolish, the frequency range of all three of the call types (Table 1–3; Fig. 1B), primarily by reducing the highest frequencies in the calls. The effects were more severe with the low cuts that selectively inactivated m. sternotrachealis (Cut XII-Low, Tables 1–3; Fig. 1C). These chicks appeared unable to produce low-intensity calls, and two of the six did not produce twitters at all (Table 2). Those that twittered did so only in the initial feeding bouts after food deprivation, a time when chicks twitter most loudly and frequently. By gently placing a finger on the throat of those chicks that failed to twitter (even though they went through the motions), one could feel the trachea moving cephalad with each attempted, but silent, "vocalization." Furthermore, slightly stronger finger pressure that prevented the movement of the trachea resulted in audible sounds with each calling movement. The calls of the chicks in which the tracheal muscles had been denervated were noticeably less loud than those of controls, and they were absent in situations in which low-intensity twitters were normally given, but no quantitative measurements were made of sound level. Durations of peeps were also reduced in the Cut-Low group. Cutting the sternotrachealis muscles (Cut Muscle, Table 1–3) had effects very similar to those of denervating them, in that it reduced the maximum frequency and the range of frequencies in all three types of calls. The results from this group and its control (Rupture Air Sac) rule out incidental damage to m. tracheolateralis or general trauma as explanations for the relative severity of

TABLE 2. Characteristics of twitter vocalizations in control and experimental groups of chicks.

	Intact Control	Operated Control	Cut XII High	Cut XII Low	Rupture Air Sac	Cut Muscles
Maximum frequency						
Mean	4,400^{a}	4,080^{a}	3,710^{a}	2,520^{b}	3,700^{a}	2,630^{b}
SE	160	150	320	100	180	80
Minimum frequency						
Mean	2,470^{a}	2,490^{a}	2,240^{a}	1,760^{b}	2,220^{a}	1,850^{b}
SE	120	200	140	90	150	60
Range (maximum–minimum)						
Mean	1,930^{a}	1,590^{ab}	1,470^{b}	760^{c}	1,480^{b}	780^{c}
SE	130	190	210	50	120	50
Number of chicks twittering	6	6	6	4	6	5

TABLE 3. Characteristics of trill vocalizations in control and experimental groups of chicks.

	Intact Control	Operated Control	Cut XII High	Cut XII Low	Rupture Air Sac	Cut Muscles
Maximum frequency						
Mean	5,060[a]	5,140[a]	3,980[b]	2,840	4,460[a]	3,180[c]
SE	150	250	200	—	180	100
Minimum frequency						
Mean	2,400[a]	2,330[a]	2,770[a]	1,880	2,470[a]	1,940[b]
SE	50	80	120	—	160	70
Range (maximum–minimum)						
Mean	2,650[a]	2,810[a]	1,710[b]	960	1,980[b]	1,240[c]
SE	150	300	110	—	100	120
Number of chicks trilling	5	6	6	1	6	4

the vocal loss after partial (Cut XII-Low) vs. total (Cut XII-High) denervation of the syringeal musculature. The reduction in frequency range of calls in the Rupture Air Sac control group, although not as severe as that in the nerve section groups, is consistent with earlier demonstrations (Gaunt et al. 1973, Youngren et al. 1974) that vocalizations depend on pressurization of the interclavicular air sac: although the incision in the neck skin was tightly closed, the air sac was not, and air could leak into the subcutaneous space to alter the dynamics of pressure changes external to the syringeal membranes.

The call of the chicks with m. ypsilotrachealis denervated, either alone or with the other two muscles, did not differ from the Operated Control and Cut XII-High groups, respectively, in any measure. Like the chicks of these two groups, however, their peeps contained significantly greater frequency excursions than those of Cut XII-Low chicks ($P = 0.007$, paired t-test). These results from chicks resemble those from adult hens in that, although m. ypsilotrachealis is often activated during vocalization (Youngren et al. 1974, Gaunt and Gaunt 1977), it does not seem to play a major role in controlling the brief types of vocalizations that we have studied.

All three types of calls were preceded by a burst of EMG activity in the abdominal (expiratory) muscle, and each amplitude-frequency peak within a trill was preceded by a well-defined burst of EMG activity that commenced 15–30 ms (mean of 40 measurements in 20 trills 22.1 ± 3.4 ms) before the sound (Fig. 2). These bursts all occurred during a single period of expiratory activity of the muscles, and activity briefly ceased between bursts within a trill. The EMG activity immediately preceding each sound, even the weakest, consistently included much larger spikes than did normal, even excited but silent, exhalation, suggesting that different sets of muscle fibers are involved in breathing and in vocalizing (fast-twitch alpha fibers for the large spikes associated with vocalizing and slower, beta fibers for the smaller spikes seen during breathing?). Because muscle fiber types are determined by their motor neurons, this suggests partial differentiation of nerve fibers for calling and for respiration.

Calls tended to occur during exhalations rather than interrupting inhalations, but several calls could be given on a single exhalation. Brackenbury (1978a: Fig. 8C) has shown the same. His figure shows strikingly that the respiratory rhythm is not reset by the calls.

Fig. 1. Audiospectrograms illustrating the three types of chick vocalizations analyzed in this investigation and the effects of the experimental treatments on them. **A.** Operated Control, skin of upper ventral neck cut and sutured. **B.** Cut XII-High, bilateral section of ramus cervicalis decendens superior of N. XII near its cephalic end. **C.** Cut XII-Low, bilateral section of ramus cervicalis decendens superior of N. XII just cephalad of its entry into the interclavicular air sac. **D.** Rupture Air Sac, incise skin, and rupture interclavicular air sac to same extent as in "E" below, as control for damage to the air sac. **E.** Cut Muscles, cut both sternotrachealis muscles free from the trachea close to their insertions on it. "Pre" columns contain examples of normal vocalizations prior to surgery, "Post" columns contain examples of vocalizations 48 h after surgery. Each horizontal calibration mark at the left of the figure represents 1 Khz (from 1 through 8).

DISCUSSION

All three types of calls were produced by the chicks after complete denervation of the tracheal muscles (Cut XII-High group), but the range of frequencies spanned by calls was significantly reduced. Selectively denervating (Cut XII-Low group) or cutting the sternotrachealis muscles had more drastic effects, eliminating all soft

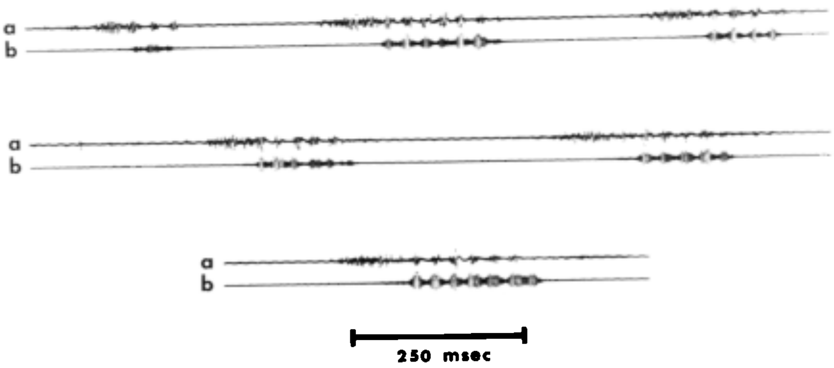

Fig. 2. Electromyograms of the expiratory muscle m. obliquus abdominis externus referenced to vocalization. A. expiratory muscle EMG. B. vocalizations (trills). Time scale, 250 mm/s.

calls and reducing the frequency ranges of the calls even more. We found the same in adults (Youngren et al. 1974); selective denervation of m. sternotrachealis altered vocalizations much more than did inactivation of both it and m. tracheolateralis together. In both young and adults reduction in frequency range was accompanied by a loss of amplitude, indicating that in chicks, as in songbirds (Greenewalt 1968, Stein 1968, Gaunt and Wells 1973), amplitude and frequency modulation are intimately linked. Our results with chicks apparently differ from those of Allen (1970, cited in Andrew 1973), who reported loss of all frequency modulation after cutting m. sternotrachealis in chicks, for we saw some modulation, although admittedly not much, after such surgery, even in the trills (Fig. 1). The literature for adult fowl is scant: aside from our report, Gross (1964) simply said that the operation failed to silence chickens, and Brackenbury (1978a) that "In their essential features, clucking and crowing remained unchanged." Miskimen (1951) reported that her two cocks eventually crowed loudly but with altered sound.

We interpret these results with domestic fowl to indicate that the coordinated actions of the extrinsic syringeal muscles play an important facilitatory role in the production and modulation of the birds vocalizations. The importance of their coordination is most apparent in the greater vocal losses in Cut XII-Low and Cut Muscle groups than in the Cut XII-High group. In addition, air pressure events controlled primarily by the expiratory musculature and pressure-flow dynamics of the system are sufficient both to produce and to modulate amplitudes (and so frequencies) of sounds in both chicks and adults.

This conclusion is strongly supported by our findings (1) that recognizable trills persist after denervation of the extrinsic syringeal muscles of chicks, and (2) that the expiratory muscles produce separate EMG bursts for each sound peak of a trill. Both audiospectrographs and oscillograph records show that a trill is a continuous sound, not a series of sounds separated by brief silent intervals as Canary (*Serinus canaria*) trills seem to be (Calder 1970). This suggests that chicks produce trills by superimposing brief, added expiratory muscle pulses (whose effects are reinforced by activities of the extrinsic syringeal muscles) to an exhalation and not by "mini-breaths" as proposed by Calder (1970) for Canary song. They thus seem to conform to the pulsatile input model (No. 4) of Gaunt et al. (1976).

The average rate of pulsing that we measured in 16 trills was 40.3 ± 6.8 (maximum 63) pulses/s. This is very close to the rate of trills in mini-breaths (37/s) that Calder (1970) reported for his Canaries and to the pulse rate of 52/s that Brackenbury (1978b) observed in the songs of *Locustella.* It is far higher than the frequency of panting, 6.25/s, that the same author has reported for a 15-day-old chick (Brackenbury 1978a). Clearly, pulsatile contractions of the respiratory muscles can play an important part role in amplitude modulations of the vocalizations of both passerine and nonpasserine birds up to repetition rates of 40–60 Hz.

Although the respiratory and vocal systems are linked to the extent that they use the same peripheral apparatus for moving air and are further related by the fact that vocalizations occur only on exhalations, their neuromuscular controls differ in several important ways. Vocalizations do not result simply from sudden, forceful exhalations, even at the level of abdominal muscle control. Our EMG data on chicks hint that different types of muscle fibers, and thus of motor neurons, may be involved in ventilatory and vocal exhalations (Fig. 2). Air-sac pressure records of both Gaunt et al. (1976: Fig. 4) and Brackenbury (1978a: Fig. 8) show that, at least some times, ventilatory rhythms are not reset even by multiple, rapidly repeated calls. This implies the existence of at least two pattern generators capable of producing independent rhythms.

Comparison of denervating and of severing m. sternotrachealis in domestic fowl and in other species suggests some basic similarities as well as some striking differences. Denervating the syrinx produces more severe vocal deficits in adult songbirds than in (infant) chicks (Nottebohm 1971, Peek 1972, Smith 1976), but the degree of loss reported in the latter ranges from the "virtually aphonic" of Nottebohm and the "mute" of Peek to "species atypical sounds" of Smith. Brockway (1967) also reported loss of species typical sounds but not muting after denervation of the syrinx in Budgerigars (*Melopsittacus undulatus*). In adult fowl, sectioning the nerves has had little effect on vocalizations (Gross 1964, Brackenbury 1978a). Clearly, denervation of syringes with intrinsic musculature more seriously impairs their capabilities than it does those of chickens, which lack intrinsic muscles. Ability to make vocal sounds persists even in some songbirds (apparently fairly loud ones in Smith's birds), suggesting a difference in degree rather than in kind from chickens.

Some role for the extrinsic muscles in songbirds is suggested by the loss of song that Miskimen (1951) reported after cutting the sternotrachealis muscles in four Cardinals (*Cardinalis cardinalis*), but Smith (1977) reported normal songs in three species of songbirds after 2 weeks for recovery. His results clearly show that the muscles are not essential for loud sounds, a result very similar to those with cock crows. Our results with chicks and adult fowl, however, suggest that the softer vocalizations of songbirds might be more severely affected: the lower air pressures associated with these softer calls would be less likely able to bow the vocal membranes into the air stream sufficiently to produce sound. This would be consistent with the demonstrated roles of the extrinsic muscles in other forms and with their persistence in song birds.

In conclusion, we suggest that the vocal mechanisms of songbirds share the basic respiratory-pump and extrinsic syringeal muscle controls that are present in chickens. Superimposed upon this, they have evolved further syringeal muscles and structures that seem to increase greatly their vocal efficiency (Gaunt and Wells 1973, Brackenbury 1979) and range of frequencies (Greenewalt 1968).

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