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Selective Laryngeal Neurotomy and the Control of Phonation by the Echolocating Bat, *Eptesicus*

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Summary. 1. The effect, on the subglottic pressure and on the emitted orientation sounds, of selectively cutting nerves to various laryngeal muscles of the Big Brown Bat, *Eptesicus fuscus*, was studied.

2. Bilateral inferior laryngeal neurotomy caused no change in the repetition rate, duration, initial frequency or bandwidth of downward sweeping frequency modulated (FM) pulses, but after this treatment mean subglottic pressure at pulse onset was lowered 8 to 16 cm H_2O and the mean peak pulse intensity was reduced 4 to 5 dB. The relationship of subglottic pressure to the bandwidth of the FM sweep was also altered (Table 1, Figs. 1, 2, 3).

3. Inferior laryngeal neurotomy also caused the bat to produce atypical rising FM pulses which began at about 20 kHz and swept upward almost one octave (Table 1, Fig. 2).

4. Total bilateral superior laryngeal neurotomy eliminated most of the FM and reduced the fundamental frequency to 7.9 kHz with multiple harmonics (Fig. 4). Pulse duration became highly variable and the peak sound pressure level (SPL) dropped 7 to 13 dB (Table 2). The correlation between subglottic pressure and pulse SPL was also eliminated.

5. Bilateral section of only the caudal branch of the superior laryngeal nerve reduced the fundamental frequency of the pulses to 10 kHz and eliminated most of the frequency modulation. Pulse SPL was reduced about 10 dB, but pulse onset subglottic pressure remained correlated with the maximum SPL (Table 2).

6. Paralysis of the tongue by bilateral section of the hypoglossal nerves distal to the thyrohyoid twigs had no detectable effect on the downward sweeping FM pulses, but caused the bats to also emit long duration pulses whose frequency rose and fell in a sinusoidal fashion (Figs. 5, 6).

7. The muscular control of pulse frequency and intensity is discussed in the light of these data. The aberrant rising FM after section of the inferior laryngeal nerves and sigmoid pulses after hypoglossal neurotomy can be understood as the result of timing errors in the opening and/or closing of the glottis relative to the contraction-relaxation cycle of the cricothyroid muscles.

Abbreviations: *FM* frequency modulation; *ILN* inferior laryngeal nerve; *SLN* superior laryngeal nerve

Introduction

We have recently investigated the role of subglottic pressure in controlling the intensity and other properties of the sonar pulses emitted by echolocating bats which have their laryngeal innervation intact (Fattu and Suthers 1981). We here describe the effect of selective laryngeal neurotomy on these ultrasonic vocalizations and on the associated changes in subglottic pressure. These experiments provide further information on the neural and muscular control of echolocative signals in the Microchiroptera. Previous authors have also used selective neurotomy of laryngeal muscles to investigate their role in the production of orientation sounds by bats, but they did not attempt to measure subglottic pressure or sound intensity.

The larynx of vespertilionid bats is adapted for the production of brief ultrasonic orientation pulses which are emitted at a high intensity and repetition rate during echolocation. The vibratory elements generating these sounds consist of thin membranes situated on the vocal, and perhaps the ventricular, folds which delimit the laryngeal ventricle and are surrounded by a massive muscular and cartilaginous framework. The tension on these membranes, and hence the frequency at which they vibrate, is controlled by contraction and relaxation of the hypertro-

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phied cricothyroid muscles (Griffin 1958; Novick and Griffin 1961; Suthers and Fattu 1973).

The innervation of the microchiropteran larynx appears to be similar to that of most other mammals (Griffin 1958; Novick and Griffin 1961; Quay 1970; Henson 1970; Griffiths 1976). Its intrinsic muscles are supplied by one of two nerves: the superior laryngeal nerve (SLN) or the inferior laryngeal nerve (ILN), respectively. The superior laryngeal nerve, a branch of N X, innervates the cricothyroid muscle by way of several small twigs. Although section of the SLN has little effect on normal speech in man, in bats it results in a marked reduction of pulse frequency and loss of frequency modulation (FM) (Griffin 1958; Novick and Griffin 1961). The inferior laryngeal nerve, also a branch of N X, innervates the other intrinsic laryngeal muscles. Intrinsic laryngeal muscles, particularly the cricothyroid, thyroarytenoid and cricoarytenoid muscles, are richly innervated in *Myotis, Rhinolophus* (Michel 1961) and *Tadarida* (Blevins and Tillman 1967; Blevins 1970). No communicating fibers were found between the SLN and ILN in *Pteropus* or *Nyctalus* (Bowden and Scheuer 1961). Although the muscles innervated by the ILN are important in the sound production of many mammals, their function in bats is unclear since their paralysis is reported to have little effect on ultrasound production (Griffin 1958; Novick and Griffin 1961). Rübsamen (1980) monitored bursts of impulse traffic in the ILN of *Rhinolophus* during respiration and phonation which he believes reflect glottal abductor and adductor activity.

Some extrinsic laryngeal muscles are served by the hypoglossal nerve (N XII) which passes twigs to the thyrohyoid muscle before innervating the tongue musculature (Sprague 1943; Novick and Griffin 1961; Feild and Holbrook 1969). Paralysis of the tongue by hypoglossal section is accompanied by an occasional increase in pulse duration (Novick and Griffin 1961).

Materials and Methods

The detailed methods of sound and subglottic pressure measurement are presented in Fattu and Suthers (1981) and will only be summarized here. Surgical procedures were performed under ether anesthesia. The larynx was exposed through a ventral skin incision by blunt dissection. Subglottic pressure was measured via an 18 gauge stainless steel T-cannula surgically implanted in the trachea and connected to a pressure transducer (Pitran model PT-H2 MO4) with a high frequency response. Sound pressure level (SPL) (re 2×10^{-5} N/m^2) was measured 15 cm in front of the bat's mouth by a calibrated condenser microphone (Brüel and Kjaer, one quarter inch diameter, model 4135). Sound and subglottic pressure were recorded on an analog magnetic tape recorder (Precision Instrument Co., model 6204) and later analyzed on a PDP-12 digital computer and Kay 6061B Sonagraph.

Vocalizations in cannulated, restrained bats were either spontaneous or induced by electrical brain stimulation following the technique of Suga and Schlegel (1972). Although all bats with tracheal cannulae were prepared for brain stimulation, i.e., restrained in head-holding devices with their inferior colliculi exposed to provide access for stimulating electrodes, electrical stimulation was necessary to induce vocalization only after complete SLN and/or hypoglossal neurotomy. Animals with ILN neurotomy or section of the posterior branch of the SLN vocalized spontaneously.

Selective Neurotomy. Two millimeter segments of the ILN immediately posterior to the cricoid cartilage were removed bilaterally in two bats. In two other bats, surgical silk ties were carefully placed loosely around these nerve bundles. After control measurements were obtained on these animals, they were lightly anesthetized and the nerves were severed by pulling the ties. Vocalizations and subglottic pressures were again recorded approximately one hour after severing the nerve with the silk ties. Superior laryngeal nerves were sectioned immediately distal to their exit from the vagus nerves. Care was taken not to sever the accompanying blood vessels. In one bat, only the caudal branch of the SLN was severed bilaterally. Hypoglossal neurotomy was performed bilaterally immediately distal to the nerve twig supplying the thyrohyoid muscle. Approximately 24 to 48 h were permitted for recovery, except in the case of two bats with acute ILN lesions. All lesions were confirmed by a post-mortem dissection.

Results

Bilateral Inferior Laryngeal Neurotomy

The ILN innervates all of the intrinsic laryngeal muscles except the cricothyroids. Since these muscles include the glottal adductors and abductors, it is not surprising that respiratory patterns after the section of this nerve suggest increased airway resistance. In normal bats, respiration is largely diaphragmatic and visible as abdominal and flank movements with little thoracic motion. After bilateral inferior neurotomy, a respiratory pattern with supraclavicular depression and costal margin flaring on inspiration, as well as excessive sternal depression on expiration, was observed. Such animals, however, remained otherwise healthy in the laboratory for periods of up to two months.

After inferior laryngeal neurotomy, three of four bats continued to spontaneously produce downward sweeping FM pulses, but one of these animals did not vocalize after insertion of the tracheal cannula.

Typical downward sweeping orientation pulses emitted by one bat, while in the experimental apparatus with the tracheal cannula in place, immediately prior to neurotomy are shown in Fig. 1. Figure 2 shows ultrasonic pulses emitted by the same individual after bilateral inferior neurotomy. Downward FM pulses (e.g., Fig. 2, pulses 2, 3, 5, 6, and 8) are still the most common vocalization, but many pulses composed of upward FM (e.g., Fig. 2, pulses 1, 4, and 7) are also emitted.

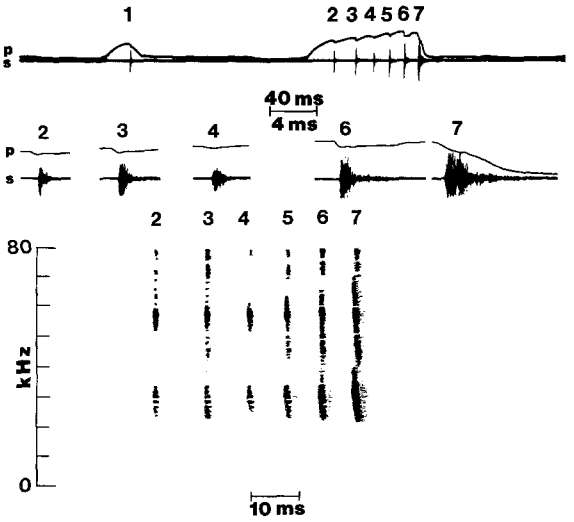

Fig. 1. Pulses emitted spontaneously by bat B and concurrent changes in subglottic pressure before inferior laryngeal neurotomy. No allowance has been made in this, or subsequent figures, for a 0.5 ms delay in the arrival time of sound at the microphone compared to the subglottic pressure. *Upper two traces:* Sequence of 7 pulses (*s*) and subglottic pressure (*p*). *Lower two traces:* Selected pulses and subglottic pressure on a time base expanded 10×. *Bottom:* Sonagrams of selected pulses

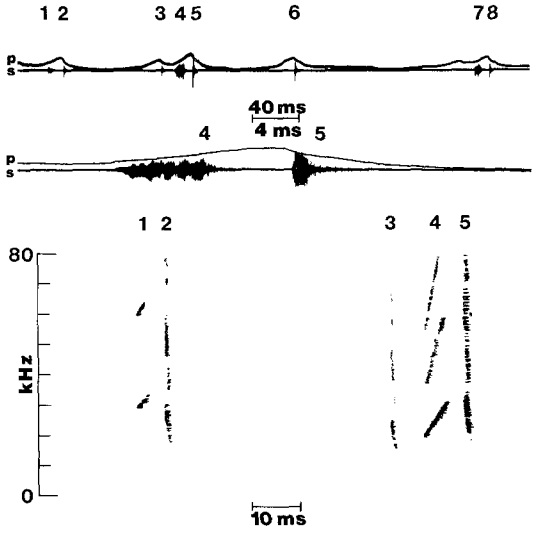

Fig. 2. Pulses emitted spontaneously by bat B and concurrent subglottic pressure after bilateral inferior laryngeal neurotomy. Rising FM pulses often precede the characteristic downward sweeping FM pulses. See legend of Fig. 1 for further explanation

Effect on Downward Sweeping FM Pulses. A quantitative analysis of a number of downward sweeping FM pulses emitted before or after bilateral section of the inferior laryngeal nerves revealed no significant change in pulse duration, the initial frequency or the bandwidth of the fundamental FM sweep as a result of this surgery (Table 1). Neither was there a significant effect on pulse repetition rate.

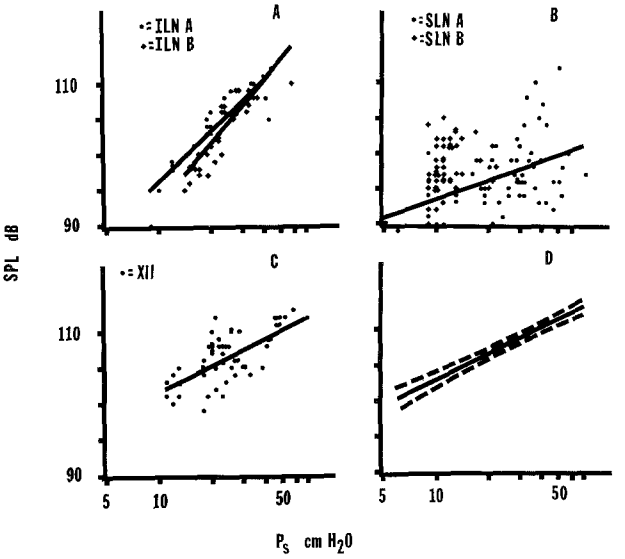

Fig. 3A–D. Maximum pulse sound pressure level as a function of subglottic pressure at pulse onset. **A** Bats A and B after bilateral inferior (=recurrent) laryngeal neurotomy. **B** After bilateral section of caudal branch of the superior laryngeal nerve (SLN A) and after total bilateral section of superior laryngeal nerve (SLN B) **C** After bilateral section of hypoglossal nerve distal to thyrohyoid twig. **D** Regression line and 95% confidence intervals for pooled data from eight bats with their laryngeal innervation intact (from Fattu and Suthers 1981)

Section of these nerves did, however, lower the mean peak intensity of the orientation pulses about 4 to 5 dB. Mean subglottic pressure at pulse onset was also 8 to 16 cm H_2O below that of the controls (Table 1). The relationship of pulse onset subglottic pressure to the maximum SPL after ILN section is plotted in Fig. 3A. In this case the control data (Fig. 3D) are based on the mean values of eight intact *Eptesicus* studied under identical conditions by Fattu and Suthers (1981). The individual plots for each of these eight control bats are given in Fig. 3A–E of that paper, in which bat A is no. 7 and bat B is no. 6.

A comparison of Fig. 3A and D in the present paper shows that in the case of bat A neurotomy caused the regression line to rotate counterclockwise around a point representing about 40 cm H_2O subglottic pressure. At lower subglottic pressures the sound pressure level was below that of the controls, whereas at high subglottic pressures it was above the controls, although the number of pulses at these high pressures is small. Unfortunately the situation is less clear for bat B, since it was unique among the control animals in lacking a significant correlation between peak SPL and the onset of subglottic pressure (Fattu and Suthers 1981). The correlation between these two variables became highly significant after neurotomy,

Table 1. Effects of bilateral inferior laryngeal neurotomy

Experiment	No. of vocalizations[a]	Fundamental frequency at pulse onset (kHz)[b]	Frequency sweep of fundamental (octaves)[b]	Sound pressure level (dB re 2×10^{-5} N/m^2)[b]		Pulse duration (ms)[b]	Subglottic pressure at pulse onset (cm H_2O)[b]	Absolute subglottic pressure change during pulse (cm H_2O)[b]	Exponent of subglottic pressure function $SPL_{max} \propto P_s^{x\,c}$
				Maximum	Mean				
Bat A control	49	23.4±5.2 (16–48)	0.9±0.3 (0.3–1.4)	109±4 (99–116)	99±3 (93–103)	3.5±1.1 (1–5)	32.5±12.7 (9–60)	22.2±10.2 (5–46)	0.79±0.10
Bat A post-neurotomy falling FM	20	25.1±7.8 (16–40) NS	1.0±0.4 (0.3–1.7) NS	104±6 (95–112) $P<0.01$	97±5 (88–104) $P<0.05$	4.8±3.0 (1–15) NS	24.3±11.7 (9–45) $P<0.01$	12.2± 6.9 (2–23) $P<0.001$	1.20±0.13
Bat B control	24	33.3±6.1 (25–48)	1.0±0.2 (0.5–1.4)	107±3 (101–111)	97±3 (92–101)	3.3±1.5 (1–6)	41.3± 8.6 (28–55)	12.6±10.3 (1–38)	0.51±0.30
Bat B post-neurotomy falling FM	31	33.8±7.7 (23–49) NS	1.0±0.3 (0.3–1.5) NS	103±5 (95–110) $P<0.01$	97±3 (92–104) NS	2.9±1.4 (1–7) NS	24.9± 9.2 (14–58) $P<0.01$	8.4± 4.8 (2–23) NS	1.47±0.14
Bat B post-neurotomy rising FM	14	20.0±2.9 (17–28)	0.7±0.3[d] (0.2–1.4)	101±4 (95–107)	96±3 (91–101)	7.5±4.1 (2–17)	11.6± 2.8 (8–17)	9.5± 4.9[d] (3–17)	

[a] All vocalizations spontaneously produced
[b] Mean ± standard deviation with range in parentheses. Probability that $\bar{x}$ post-neurotomy $\neq \bar{x}$ control
[c] Exponent ± standard error
[d] Value increases during rising FM pulses

however, and the slope of the postoperative regression line is steeper than predicted by the control data.

The relationship between FM and subglottic pressure was also altered by inferior laryngeal neurotomy. The subglottic pressure of bat A dropped an average of about 10 cm H_2O less after section of these nerves than it did during comparable frequency sweeps when the ILN were intact ($P<0.01$). A similar tendency was present in bat B, but was not statistically significant. Nevertheless, there was a significant positive increase in the slope of the regression line relating the bandwidth of the fundamental to the concomitant drop in subglottic pressure.

Upward Sweeping FM Pulses. In addition to the descending FM pulses described above, all three of the bats which continued to vocalize after bilateral inferior laryngeal neurotomy also spontaneously produced atypical rising FM pulses (Fig. 2) quite unlike the typical echolocative pulses of intact animals. Rising FM pulses were emitted during flight by bats without a tracheal cannula, as well as by cannulated bats restrained for recording.

The characteristics of these rising FM pulses are also summarized in Table 1, although the sample size is too small to permit statistical comparison with the downward sweeping vocalizations. The duration of rising FM pulses was approximately twice that of downward FM pulses emitted either before or after neurotomy, despite the fact that the rising pulses swept through a narrower range of frequencies. Rising FM pulses typically began at about 20 kHz and swept linearly upward a little less than one octave. Their average peak intensity was about 6 dB below that of declining FM pulses before neurotomy and slightly below that of the post-neurotomy downward FM pulses. The mean SPL is comparable to that of declining FM pulses. Subglottic pressure at pulse onset ranged between 3 and 17 cm H_2O and rose gradually, rather than fell, during the course of the pulse (Table 1).

Superior Laryngeal Neurotomy

Exposure of SLN requires more difficult microsurgery entailing some mortality. The presence of the tracheal cannula and pressure transducer compounds this difficulty, making pre-neurotomy subglottic pressure measurements impracticable. Effects of SLN lesions on subglottic pressure were therefore deduced by

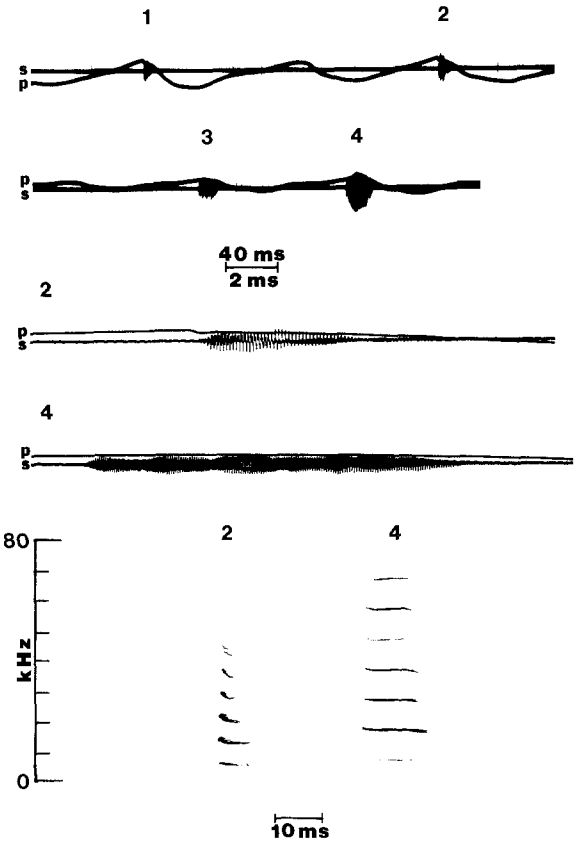

Fig. 4. Subglottic pressure and pulses emitted after total bilateral superior laryngeal neurotomy. See legend of Fig. 1 for further explanation

comparing the post-lesion data to those from nine intact cannulated bats (Fattu and Suthers 1981).

The superior laryngeal nerve innervates the hypertrophied cricothyroid muscles. Total bilateral section of this nerve caused important changes in the properties of the emitted vocalizations and the associated subglottic pressure. Data could be obtained from only two of several bats upon which an attempt was made to combine this surgery with subglottic pressure measurements.

Total Superior Laryngeal Neurotomy. After total denervation of the cricothyroid muscles, a bat with an implanted tracheal cannula emitted cries having a fundamental frequency of 7.9 kHz compared to mean onset frequencies of from 18.4 to 33.3 kHz for individual control bats (Table 2). Multiple harmonics were present and there was generally negligible frequency modulation, although occasionally rising or declining frequency sweeps over a range of 9 to 12 kHz were observed. Pulse duration was highly variable, ranging from 2 to 54 ms. Peak SPL was 7 to 13 dB below that of the controls (Fig. 4, Table 2).

Total bilateral superior laryngeal neurotomy abolished any significant correlation between the pulse onset subglottic pressure and the peak intensity of the pulse (Fig. 3B). It also eliminated a correlation, present in intact bats, between the frequency range of the FM sweep and concurrent change in subglottic

Table 2. Effects of bilateral superior laryngeal neurotomy

Experiment	No. of vocalizations	Fundamental frequency at pulse onset (kHz)[d]	Frequency sweep of fundamental (octaves)[d]	Sound pressure level (dB re 2×10^{-5} N/m^2)[d]		Pulse duration (ms)[d]	Subglottic pressure at pulse onset (cm H_2O)[d]	Absolute subglottic pressure change during pulse (cm H_2O)[d]	Exponent of subglottic pressure function $SPL_{max} \propto P_s^{x}$[e]
				Maximum	Mean				
Controls[a]	288	18.4±3.8 to 33.3±6.1	0.4 ±0.3 to 1.0 ±0.3	105±4 to 111±2	97±3 to 104±2	2.4± 0.8 to 33.0±18.1	12.5± 4.2 to 43.4± 8.8	6.2± 3.1 to 22.9±10.5	0.40±0.10 to 1.36±0.19
Total superior laryngeal neurotomy[c]	47	7.9±1.2 (7–12) $P<0.001$	0.02±0.08 [(−0.2)–0.2][f] $P<0.001$	98±4 (87–105) $P<0.001$	93±3 (88–102) $P<0.001$	19.3±12.8 (2–54)	12.1± 3.3 (9–22) NS	4.2± 3.2 [(+3)–16][g] $P<0.05$	0.37±0.24 NS
Caudal branch superior laryngeal neurotomy[b]	59	10.0±2.0 (8–15) $P<0.001$	0.1 ±0.2 (0–0.8) $P<0.001$	97±5 (90–112) $P<0.001$	92±4 (87–106) $P<0.001$	11.0± 8.8 (2–32)	28.9±14.4 (5–72)	17.4±11.5 [(+13)–51][h]	0.43±0.11

[a] Lowest and highest mean value±standard deviation for 9 bats. See Fattu and Suthers (1981)
[b] Spontaneously emitted pulses
[c] Pulses elicited by brain stimulation
[d] Mean±standard deviation with range in parentheses. Probability that $\bar{x}$ post-neurotomy $\neq$ lowest mean of 9 control bats if post-neurotomy value is less than lowest control mean
[e] Exponent±standard error
[f] Rise of 0.2 octave to fall of 0.2 octave
[g] Rise of 3 to drop of 16
[h] Rise of 13 to drop of 51

pressure. Peak subglottic pressures were in the low range of the control animals, the mean after neurotomy being 12.1 cm H_2O compared to individual means of from 12.5 to 43.4 cm H_2O in control bats (Table 2).

Partial Superior Laryngeal Neurotomy. The superior laryngeal nerve divides into two branches just before reaching the larynx. The rostral branch is believed to be sensory and the caudal branch, which subdivides further on the surface of the cricothyroid muscle, is considered to contain the motor fibers. Bilateral section of the caudal branch produced a drop in vocal frequency and intensity, and an increase in pulse duration, similar to that caused by total superior laryngeal neurotomy (Table 2).

After this surgery, however, the bat was still able to achieve relatively high subglottic pressures during phonation. These pressures lie well within the range of those recorded from control animals. Pulse onset subglottic pressure was also still significantly correlated with the maximum SPL. The slope of the regression line is similar to that of the control bats, but shifted downward so that for a given subglottic pressure, the peak pulse intensity is about 10 dB lower than that of the controls (Fig. 3B, Table 2). Nevertheless, some pulses had a peak intensity up to 112 dB and were accompanied by subglottic pressures as high as 72 cm H_2O.

Frequency modulation was greatly reduced by section of the caudal branch of the SLN, but pulses typically exhibited slightly more FM than after total SLN section. This residual FM may be due to a passive stretching of the vocal membranes by the higher subglottic pressure that exists when only the caudal branch is cut. The bandwidth of the FM sweep also remained positively correlated with the absolute change in subglottic pressure occurring during the sweep. However, for any given subglottic pressure change, the extent of the frequency sweep was approximately 0.5 octave less than in the control bats.

Section of Both Inferior and Superior Laryngeal Nerves

One *Eptesicus* underwent total inferior laryngeal neurotomy subsequent to total superior laryngeal neurotomy. As expected, and in spite of the use of brain stimulation, this bat was silent. Subglottic pressures were less than two cm H_2O. Respiration was slower and deeper than normal.

Hypoglossal Neurotomy

The tongue of *Eptesicus* appears to move in synchrony with the emission of sonar pulses. The hypoglossal nerves were cut in order to determine if these movements are necessary for normal phonation. Bilateral hyoglossal neurotomy distal to the thyrohyoid twigs paralyzes the tongue. This procedure is also associated with high mortality, probably as a consequence of an impaired ability to swallow salivary secretions. Therefore, the same nine *Eptesicus* studied by Fattu and Suthers (1981) also served as controls for these animals.

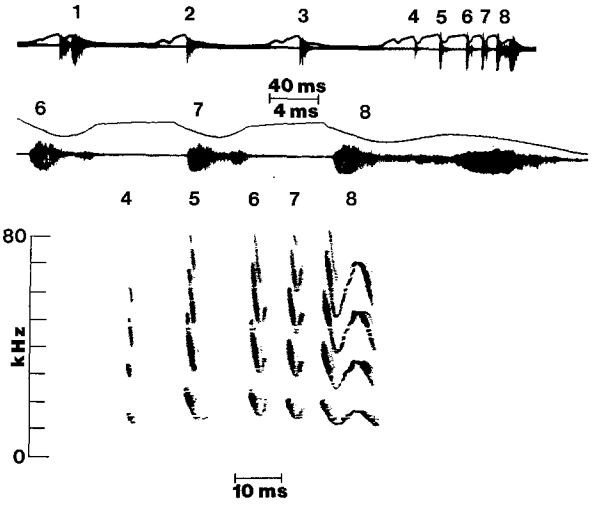

Fig. 5. Subglottic pressure and pulses emitted after bilateral hypoglossal neurotomy distal to the thyrohyoid twigs. Pulse 8 is characteristic of sigmoid pulses in which pulse frequency and intensity closely approximate changes in subglottic pressure. See legend of Fig. 1 for further explanation

Sigmoid Pulses. Although bats continued to produce downward sweeping FM pulses after neurotomy, they also emitted long duration, continuously rising and declining FM ultrasonic vocalizations (Fig. 5). Because of this roughly sinusoidal pattern of continuous frequency modulation, these vocalizations are called 'sigmoid pulses'. Sigmoid pulses constituted 8% of the vocalizations elicited by brain stimulation from one animal and 20% of the vocalizations from another bat. The mean duration of 32 such pulses was 26.8 ± 11.4 ms, much longer than typical orientation pulses. Pulses of this form have occasionaly been recorded from control bats vocalizing spontaneously (e.g., Fattu and Suthers 1981, Fig. 1). During sigmoid pulses there is simultaneous modulation of frequency, intensity and subglottic pressure with these three parameters generally increasing and decreasing together (Fig. 6). Sigmoid pulse intensities paralleled subglottic pressure, and peak values were comparable to those of some control bats. The SPL 0.2 ms after pulse onset was about 90 dB at a subglottic pressure of about 15 cm H_2O. Mean speak SPL's were 110 dB at subglottic pressures around 20 to 25 cm H_2O.

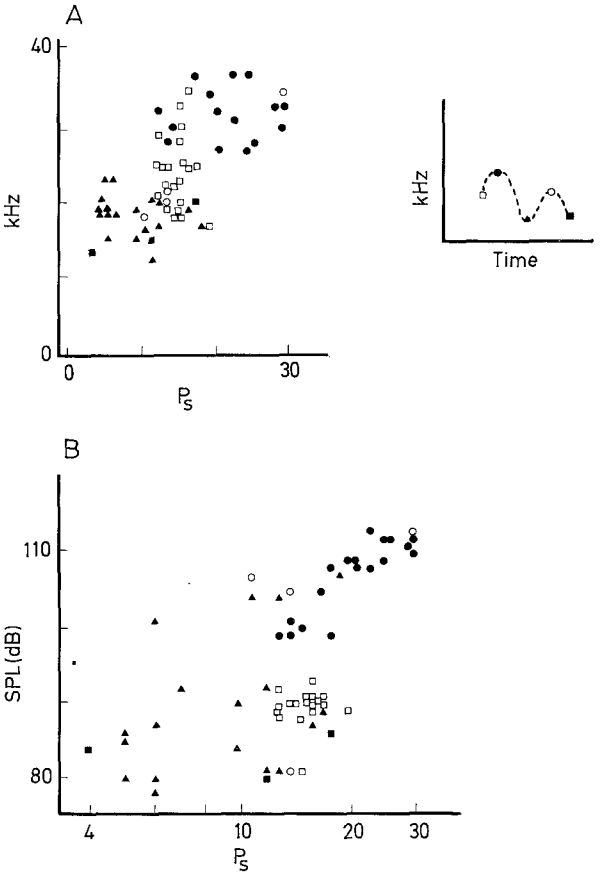

Fig. 6. The relationship between subglottic pressure of sigmoid pulses emitted after hypoglossal neurotomy and their frequency (**A**) and sound pressure level (**B**). *Inset:* Points at which frequency, intensity and subglottic pressure were measured. Sigmoid pulses were initiated during either a rising (*open square*) or declining (*filled circle*) frequency modulation

Downward Sweeping FM Pulses. Downward sweeping FM pulses were more common than sigmoid pulses. Analysis of 52 of these vocalizations failed to reveal any significant differences from similar pulses emitted by the control group of bats. The pulse SPL retained a significant correlation with subglottic pressure. The maximum SPL was proportional to the pulse onset subglottic pressure raised to a power of 0.64 ± 0.09 (Fig. 3).

Discussion

Considerable caution is necessary when interpreting the results of experiments such as these which attempt to understand a motor activity by selectively denervating certain muscles, or groups of muscles, which contribute to the total motor pattern. This is particularly true in the case of ILN section, since this nerve innervates several pairs of intrinsic laryngeal muscles, some of which – such as the glottal adductors and abductors – have antagonistic actions. Furthermore, it is not known to what extent the animal may be able to use the remaining muscles to compensate for a function normally performed by the paralyzed muscle. Nevertheless, useful information can be obtained by such experiments. Although ILN section gives no direct information about the role of specific muscles, it does provide an indication of the net role in phonation of intrinsic muscles other than the cricothyroids and helps to clarify the functions of the cricothyroid muscles themselves. Interpretation of our data is also aided by knowledge of the effect of each treatment on subglottic pressure.

In an intact bat, phonation is preceded by closing the glottis and initiating expiratory effort which causes subglottic pressure to rise. At some point increased subglottic pressure and/or relaxation of the glottal adductor muscles allows the glottis to open slightly, and air flowing past the thin vocal membranes presumably initiates Bernoulli forces which cause them to vibrate. The frequency of vibration is determined by the tension exerted across the vocal membranes, which have no intrinsic muscles. Phonation is terminated by further opening the glottis, thus abducting the vocal membranes and reducing subglottic pressure.

Control of Intensity

Our data indicate that both the cricothyroid muscles (served by the SLN) and the other intrinsic laryngeal muscles (served by the ILN) play a role in controlling the SPL of the emitted orientation sound.

Effect of ILN Section. Most of the effects of bilateral section of the inferior laryngeal nerve can be attributed to the resulting paralysis of muscles abducting or adducting the glottis. The exaggerated respiratory effort which is evident in such animals indicates an increased resistance of the upper airway due to incomplete abduction of the glottis. The abductive role of the muscles served by the ILN is even more critical in *Rhinolophus*, where bilateral section of this nerve causes the bat to suffocate (Schuller and Suga 1976).

In *Eptesicus*, paralysis of the glottal adductors served by the ILN causes the glottis to open at a subglottic pressure about 8 to 16 cm H_2O below normal. This results in a 4 to 5 dB drop in the maximum SPL of the emitted orientation pulses. Fattu and Suthers (1981) found that the SPL of intact *Eptesicus* is approximately linearly proportional to the onset subglottic pressure (i.e., SPL proportional to subglottic pressure raised to an exponent of about 1). After neurotomy the slope of the regression line relating these two variables became steeper. The subglottic pressure exponent increased from 0.8 to 1.2 in the case of bat A and from a statistically insignificant 0.5 to a significant 1.5 for bat B.

Post-neurotomy pulses emitted by bat A having onset subglottic pressures below about 40 cm H_2O were at a lower SPL than were pre-neurotomy pulses emitted at similar subglottic pressures. This may result from an incomplete adduction of the glottis, allowing some unmodulated air (i.e., air not set into vibration by the vocal membranes) to escape. The steeper slope of the regression line after neurotomy may be due to more complete modulation of the air at higher subglottic pressures. As increasing subglottic pressure causes the rate of airflow over the vocal membranes to increase, the Bernoulli forces acting on the membranes also increase, resulting in vibrations of larger amplitude. As this happens, the proportion of unmodulated air passing through the larynx is gradually reduced and may return to normal when the subglottic pressure reaches about 40 cm H_2O.

Effect of SLN Section. Analysis of orientation sounds emitted after bilateral SLN section shows that, in addition to being a major regulator of frequency, the cricothyroid muscle of *Eptesicus* is also involved in regulating pulse intensity, a function which it shares with other intrinsic muscles. Pulse intensity is lowered on the order of 10 dB by SLN section. This is accompanied by a large decrease in subglottic pressure, estimated to be about 20 cm H_2O, indicating that these muscles also exert considerable adductive action on the glottal stop.

It is generally assumed that the rostral branch of the SLN contains sensory fibers and the caudal branch contains motor fibers, although this assertion appears to lack physiological confirmation in bats. Section of both these branches abolishes any consistent relationship between sound pressure level and subglottic pressure. Section of only the caudal branch results in a similar drop in SPL, but subglottic pressure remains higher and the correlation of peak SPL with onset subglottic pressure is retained. Novick and Griffin (1961) reported that section of only the sensory branch of the SLN had no obvious effect on the emitted vocalizations.

Control of Sound Frequency

Section of both ILN's has no significant effect on the frequency of the laryngeal generator. The occasional upward sweeping FM pulses which are produced after this treatment represent a failure in the gating, or timing of phonation (see below).

Paralysis of the cricothyroid muscles, which exert tension on the vocal membranes by flexing the cricothyroid joint, dramatically lowers the fundamental frequency of the orientation pulse, eliminates most of the FM and introduces a series of harmonic components. Similar effects have been reported by other investigators in various bats (Griffin 1958; Novick and Griffin 1961; Schuller and Suga 1976). In the constant frequency bat *Rhinolophus* the spike rate of muscle fibers during vocalization is proportional to the frequency of the CF component (Schuller and Suga 1976). Denervation of these muscles alone has a much more drastic effect on the orientation sounds than does the paralysis of all the other intrinsic laryngeal muscles. This clearly indicates the special importance of the cricothyroid muscles in echolocation.

The cricothyroid muscle is also involved in regulating vocal fold frequency in man (Shipp and McGlone 1971) and in dogs (Rubin 1963; Koyama et al. 1971). Its importance in controlling frequency increases with the frequency of the vocalizations, however. Thus paralysis of the cricothyroid muscles has little effect on normal human speech, but a great effect on high tones in singing. In squirrel monkeys, paralysis of this muscle eliminates all calls with a fundamental above 1 kHz (Jürgens et al. 1978).

The Glottal Gate: Control of Frequency Pattern and Duration

Suthers and Fattu (1973) found that the cricothyroid muscle of *Eptesicus*, which acts to increase the tension across the vocal membranes, twitches just before each orientation pulse so that the membrane tension is maximum at pulse onset and decreases during the pulse, thus creating the normal downward FM sweep. The vocal membranes thus undergo a cyclically rising and falling tension corresponding to the contractions of the cricothyroid muscles. In order to produce the downward sweeping echolocative pulses typical of most *Eptesicus* orientation sounds, the glottis must open just as the cricothyroid muscles start to relax and close before relaxation ends. The glottis thus acts to gate phonation. The type of pulse produced (e.g., rising FM, falling FM, CF, CF-FM, etc.) depends on the temporal relationship of the glottal gate to the cycle of contraction and relaxation of the cricothyroid muscles. In *Rhinolophus* and *Pteronotus*, which produce pulses that are largely CF, the electrical activity in portions of the cricothyroid muscle continues during the pulse (Schuller and Suga 1976; Suthers, unpublished data).

The possibility of such a pulse gating mechanism in bats was first introduced by Pye (1967) who used a model to show that by selectively gating portions of a simulated laryngeal waveform and tuning it through a variable Q filter representing the vocal tract, various types of sonar pulses typical of many bat species can be simulated.

Our experiments confirm the crucial importance of the timing of the glottal gate in determining the frequency pattern of the echolocative pulse and also shed some light on the muscular control of the gating mechanism.

One of the most interesting results of inferior laryngeal neurotomy is the appearance of abnormal FM pulses which sweep up instead of down. We believe these upward sweeping pulses occur because section of the inferior laryngeal nerve has eliminated some important glottal adductor muscles, permitting the subglottic pressure to occasionally force the glottis open prematurely when the cricothyroid muscles are still contracting and the tension of the vocal membranes is rising. The result is an upward sweeping FM pulse instead of a downward sweeping one. Inspection of Fig. 3 reveals that the glottis must open only very slightly during such pulses since the subglottic pressure continues to rise, indicating a high airway resistance. The glottis closes briefly, terminating the rising FM, just before the pressure reaches its peak. It then opens again to produce a normal downward FM pulse as the cricothyroid muscles begin to relax, allowing vocal membrane tension and subglottic pressure to fall.

The long duration, sigmoid pulses, which appear after bilateral section of the hypoglossal nerves distal to the thyrohyoid twig, must also represent a breakdown in the pulse gating mechanism at the level of the glottis. It is likely that normal gating requires the participation of extrinsic as well as intrinsic muscles. Proper action of the cricothyroid muscles may depend on a firm anchor via suspensory muscles to the hyoid apparatus. Kallen et al. (1968) proposed that the cricothyroid muscles may act against, and temporarily overcome, constant forces by other muscles – including the base of the tongue – which tend to anchor the thyroid cartilage to the mandible and seat the epiglottis against the oral surface of the palate.

Hypoglossal neurotomy has not been previously reported to affect pulse frequency pattern, although the occasional prolonged pulse reported by Novick and Griffin (1961) may be the sigmoid pulses we have recorded. Motta (1951–52) found obstacle avoidance was impaired in 5 out of 14 bats. He also reported that combined hypoglossal and inferior laryngeal neurotomy impaired obstacle avoidance in all individuals tested. Pharyngeal cautery has also been found to lower pulse frequency and prolong some pulses (Novick and Griffin 1961).

The tendency for pulses to be of longer and more variable duration after section of the SLN suggests that the cricothyroid muscles are also involved in the glottal gate.

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