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Subglottic Pressure and the Control of Phonation by the Echolocating Bat, *Eptesicus*

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Summary. 1. The respiratory dynamics of phonation, particularly the relationship between subglottic pressure and the sound pressure level (SPL), frequency and duration of vocalizations by nine bats (*Eptesicus fuscus*) is described.

2. Subglottic pressure rises to about 30 or 40 cm H_2O immediately prior to emission of a single FM pulse or group of pulses, respectively, and drops 5 to 20 cm H_2O during the course of each pulse (Fig. 2).

3. The maximum SPL of the echolocative pulse is positively correlated with the magnitude of the subglottic pressure at the onset of phonation in eight bats. The unusually high subglottic pressure in bats is an adaptation for the production of high intensity orientation pulses, thus increasing the range of echolocation.

4. The maximum SPL was proportional to the subglottic pressure at the pulse onset raised to a power which ranged from a mean of 0.4 to 1.36 for individual *Eptesicus* (Fig. 3 and Table 1). The approximately linear relationship between sound pressure level and onset subglottic pressure in several bats suggests the vocal tract has a minimal effect on pulse intensity.

5. Within a given vocalization, upward sweeping FM is usually associated with either constant or increasing subglottic pressure (Figs. 4 and 5), but the absolute value of the subglottic pressure at pulse onset is correlated with the initial frequency of vocalization in only four of these bats (Table 2).

6. Subglottic pressure drops most rapidly during short pulses, but during long vocalizations (exceeding 20 ms) it attains a minimum rate of decline of about 0.2 cm H_2O/ms (Fig. 6 and Table 2), which may be determined by the maximum glottal resistance at which phonation can occur.

7. The relatively non-compliant lung of *Eptesicus* (mean static compliance $= 0.021 \pm 0.002$ ml/cm H_2O) may be an adaptation for the production of the unusually high subglottic pressures needed to produce high intensity echolocative pulses.

Introduction

The insectivorous echolocating bat, *Eptesicus fuscus* (Vespertilionidae), emits high intensity, ultrasonic orientation pulses with most of their acoustic energy between 20 and 50 kHz. Downward sweeping FM pulses, having durations from less than 1 to about 5 ms, are characteristically emitted at high repetition rates of up to about 200 pulses/s when the bat is intercepting insects or negotiating obstacles. When flying several meters above the ground, longer pulses, having portions of nearly constant frequency, are emitted at lower repetition rates. Sound pressure level (SPL) several centimeters in front of the mouth may approach 120 dB re 2×10^{-5} N/m^2 (Griffin 1958; Simmons and Vernon 1971). The vocal ability of these bats poses important questions regarding the control of phonation.

Previous anatomical (Elias 1907; Griffin 1958; Griffiths 1978) and physiological (Griffin 1958; Novick and Griffin 1961; Suthers and Fattu 1973; Schuller and Suga 1976; Suthers and Durrant 1980) studies of the microchiropteran larynx have shown that the ultrasonic echolocative pulses are generated by the vibration of specialized thin membranes within the larynx, and that the cricothyroid muscles play an important role in determining the frequency of the vocalization by varying the tension on these membranes. Unlike some species, which emit constant frequency pulses, the vocal tract of bats, such as *Eptesicus*, which emit broadband, frequency modulated pulses, is broadly tuned and appears to have little effect on the spectral content of the emitted sound

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(Pye 1967; Schnitzler 1970, 1973; Roberts 1972a, 1973; Suthers and Durrant 1980).

Except for some studies on the temporal placement of vocalizations in the respiratory cycle (Schnitzler 1968; Roberts 1972b; Suthers et al. 1972), very little is known about the respiratory dynamics of phonation during echolocation. Subglottic pressure provides the driving force for phonation, so its effect on the intensity and frequency of the bat's echolocative pulses is of particular interest. In man and other mammals studied, subglottic pressure plays a major role in determining the intensity, and can also affect the frequency, of the laryngeal waveform (e.g., van den Berg 1956; Ladefoged and McKinney 1963; Koyama et al. 1969). Suthers and Fattu (1973) showed that, in *Eptesicus*, the subglottic pressure immediately prior to phonation attains values much higher than those occurring during human speech. They suggested that this unusually high pressure is necessary for the production of high intensity orientation pulses which in turn increases the range of target detection.

In this paper we examine the relationship of subglottic pressure to the SPL, frequency and duration of the echolocative pulses in order better to understand the factors which control these important aspects of the bat's vocalizations.

Materials and Methods

These experiments were performed on the big brown bat, *Eptesicus fuscus*. Surgical procedures were performed under ether anesthesia. An incision was made in the scalp, muscles were reflected, and the region over the superior and inferior colliculi was exposed. A 2 cm nail was then fastened to the skull with dental cement for later immobilization of the head. Experiments were conducted one hour after recovery from the anesthetic. Vocalizations were either spontaneous or induced by delivering brief trains of electrical pulses to the midbrain after the method of Suga and Schlegel (1972). Paired stimulating electrodes were made from electrolytically sharpened size 00 insect pins, insulated except for the tips. Electrical stimuli consisting of repetitive trains of six 0.1 ms duration pulses were delivered at the rate of two trains per s. The pulse interval within each train was 1.6 ms.

Vocalizations were measured 15 cm in front of the bat's mouth with a calibrated one-quarter inch (6.35 mm) Brüel and Kjaer condenser microphone (model 4135). The signal was amplified and recorded on a Precision Instrument Co. model 6204 tape recorder having a flat direct response from 200 Hz to 100 kHz. Sound pressure levels were measured re 2×10^{-5} N/m^2. Apparatus within 1 m of the bat was covered with one inch thick cotton batting to reduce the echoes of vocalizations.

Airway pressures during vocalization were measured with a pressure transducer (Pitran model PT-H2 M04, Stow Laboratories, Inc.) capable of an exceptionally high frequency response with small volume displacements. Subglottic pressure was measured by coupling the pressure transducer to the sidearm of a T-cannula made of 18 gauge stainless steel hypodermic tubing and inserted in the trachea 3 to 5 rings below the larynx. The total length of tubing from trachea to transducer was 1 cm. The mechanical natural frequency of the transducer alone is 150 kHz.

The 1 cm sidearm of the T-cannula substantially limited the high frequency response, however. The response of the pressure transducer with its 1 cm long probe was flat from DC to 400 Hz and remained within ± 3 dB up to about 3 kHz before rolling off. The power spectrum which was computed for the waveform of the subglottic pressure of 30 orientation pulses showed that 98% of the energy is below 200 Hz. If rapid pressure transients contain spectral components above 3 kHz they will be disproportionately attenuated. Pressure signals were amplified and tape recorded in the FM mode in which the recorder's frequency response was essentially flat from DC to 10 kHz.

Several factors which might influence the measurement of airway pressure and emitted sound were investigated. Temperature sensitivity of the pressure transducer caused its output to drift gradually when it was connected to the warm bat. During an experiment thermal drift was monitored and errors due to it were kept below 0.5 cm H_2O. The attenuation of the tube coupling the pressure transducer to the airway was measured by attaching variable lengths of closed polyethylene tubing to the sidearm of the T-cannula in three vocalizing bats. In all bats a decrease in sound pressure level acompanied increased coupler volumes. The coupler used in the following experiments had a volume of 8 mm^3, which appeared to be responsible for a 3 to 8 dB drop in emitted sound intensity. In addition, the pulse frequency of cannulated bats was somewhat lower than that of either the control animals before cannulation or of free-flying bats. Resonance of the coupling tube, closed at one end by the transducer membrane, should peak at 8,700 Hz and therefore not be a source of error in the range of interest.

Respiratory volumes and the timing of pulses in the respiratory cycle were measured with a body plethysmograph constructed from a plexiglass cylinder having a total volume of 90 ml. The bat's head protruded through an elliptical hole in a rubber dam facing which fit snugly around the animal's neck and was covered with pliable dental impression medium (Unilastic, Kerr Mfg. Co.) to reduce compliance and insure an airtight seal. Pressure changes were measured with a PT5-A volumetric pressure transducer (Grass Instrument Co.). System steady state response was linear over volume changes from 0 to 0.5 ml, which covered the range of bat tidal volumes. System frequency response, tested by a step input, was flat ± 3 dB from 0.05 to 40 Hz. The low frequency cutoff was set by a small air leak in order to compensate for temperature effects. Gas compression within the plethysmograph during respiration was computed to cause less than a 1% error in the volume measurement. The volume signal from the plethysmograph was amplified and tape recorded in the FM mode.

During an experiment 3 to 8 min of data were recorded on analog magnetic tape. These data were visually edited on an oscilloscope into several 7 s segments containing large numbers of vocalizations. The edited recordings of sound and airway pressure data were slowed on playback by a factor of 10 and were loaded onto digital magnetic tape on a PDP-12 digital computer with a sampling interval of 1.0 ms, i.e., real time sampling interval 0.1 ms.

Prior to analog to digital conversion, sound recordings were full wave rectified and passed through a third order linear-phase-shift filter (Paynter) in order to reproduce only the sound envelope (Philbrick/Nexus Research, 1968; Gottlieb and Agarwal 1970). The output of the filter has a rise/fall time of about 1 ms which is equivalent to an uncertainty of 0.1 ms in real time for pulses reproduced at one-tenth speed. This introduces negligible error since actual bat pulses have envelopes which rise to peak amplitudes at more gradual rates than did the simulated pulse.

Pulse duration as well as maximum and mean sound pressure levels were computed on the PDP-12. Mean sound pressure levels were averaged at 0.1 ms intervals. The sampling rate of the computer was too slow to analyze the frequency of the ultrasonic pulses, even when they were reproduced at one-tenth their original

speed. Fundamental frequencies were therefore determined from sonagrams (Kay Electric Co., Model 6061B Sonagraph) or from the lowest frequency component of oscillographs of their waveforms. The range of frequency sweep of the fundamental component of FM pulses was determined by measuring the initial (highest) and terminal (lowest) frequency.

Subglottic pressure, computed on the PDP-12, was correlated with the pulse onset and duration. Correction was made in computations for the 0.5 ms lag in sound transmission from the bat to microphone. Inspiratory and expiratory tidal volumes, volume changes during pulse emission, and timing of pulses in the respiratory cycle were measured from oscillographs. Statistical tests are from Ostle (1963).

In order to assess lung recoil, static lung compliance was measured in freshly excised lungs from eight bats. Lung inflation and deflation were produced over 4 min periods with a Harvard model 901 Infusion/Withdrawal pump driving a 1 ml glass syringe. Volume input was measured by a Sanborn model 7DCDT-3000 linear variable differential transformer. Lung pressures were measured via a sidearm to a Statham model PT 23AA pressure transducer. Volume and pressure signals were recorded on a Beckman Offner polygraph.

Results

Vocalizations

The *Eptesicus* used in these experiments, vocalizing spontaneously or as a result of electrical stimuli delivered to the brain, emitted a variety of ultrasonic and audible vocalizations. The most common of these consisted of a downward sweeping FM pulse similar to those utilized for echolocation by wild *Eptesicus* (Fig. 1A and B), except that under the conditions of these experiments the fundamental frequency was usually lower and the harmonics often more prominent than is characteristic of echolocative pulses emitted prior to insertion of the tracheal cannula with its pressure transducer.

At low pulse repetition rates, of about 5 to 8 per s, only a single pulse was produced during a respiratory cycle. These pulses swept downward from a starting frequency between 12 and 40 kHz (usually between 25 and 30 kHz) over a range of 0.5 to 1 octave within a pulse duration of 5 to 15 ms. Single pulses with durations as great as 20 to 70 ms were occasionally emitted.

Higher repetition rates are achieved by producing more than one vocalization per respiratory cycle. Groups of up to 10 FM pulses per breath and pulse repetition rates within groups of up to 110 pulses/s were studied. The initial frequency of pulses within groups was between 15 and 48 kHz, which is similar to that of single pulses, and swept downward over about one octave. With the exception of bat 4, most

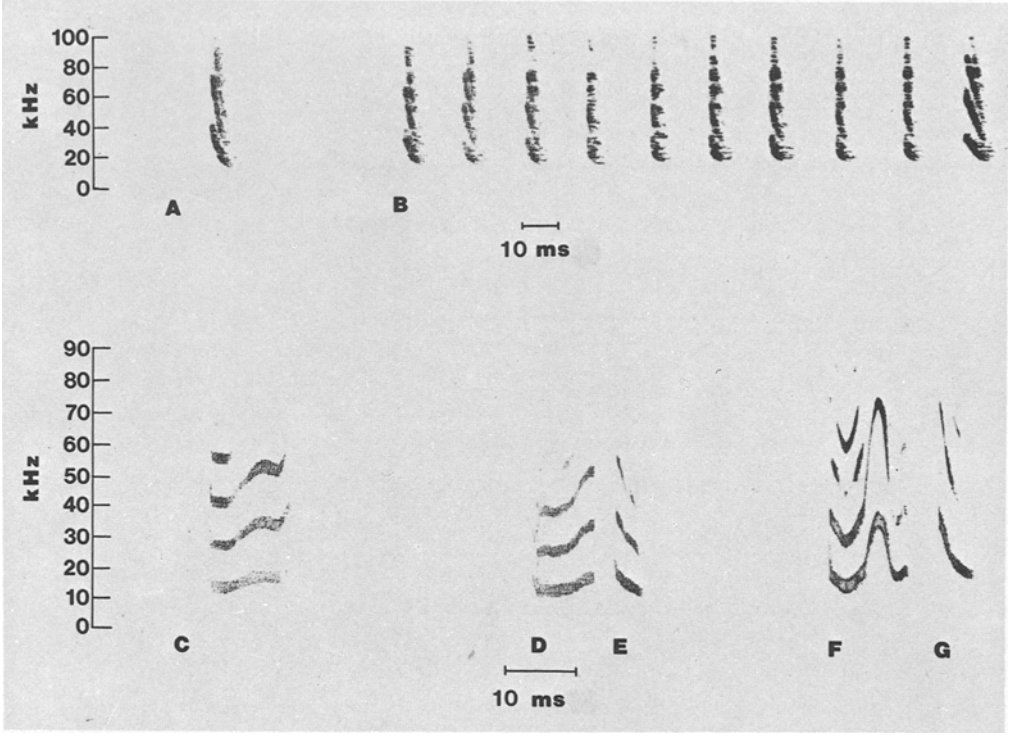

Fig. 1A–G. Sonagrams of selected vocalizations by *Eptesicus*. **A** An FM pulse emitted at a low repetition rate. **B** A high repetition rate buzz sequence of the same bat, emitted during one respiratory cycle. **C–G** Long duration vocalizations containing rising FM portions. Subglottic pressures associated with these vocalizations are shown in Figs. 4 and 6. Effective bandwidth of spectrograph filter was 900 Hz for pulses in top row and 3 kHz for vocalizations in bottom row

single pulses were elicited by brain stimulation, whereas groups of pulses were usually produced spontaneously. In bat 5 pulses were emitted both singly and in groups after brain stimulation.

Occasionally longer vocalizations were emitted (Fig. 1C–G). These had a fundamental frequency between 10 and 40 kHz and often contained both upward and downward frequency sweeps extending from one half to more than one octave. Their duration was usually greater than 15 ms and their peak SPL was typically between 105 and 110 dB.

Audible agonistic vocalizations, or 'protest cries' (see Suthers and Fattu 1973), and rapid sequences of clicks were also ocassionally emitted during these experiments, but are not included in the analysis that follows.

Subglottic Pressure During Vocalization

The relationship between subglottic pressure, SPL, pulse frequency and pulse duration was analyzed in nine *Eptesicus*.

Subglottic pressure always markedly increases prior to vocalization, typically rising to about 30 cm H_2O during the 15 to 50 ms immediately preceding the emission of a single FM pulse and to about 40 cm H_2O before emission of a group of pulses. The onset of phonation usually coincides with this peak pressure level (Fig. 2). We assume that the glottis closes just prior to phonation allowing subglottic pressure to increase. At the onset of phonation the glottis opens, allowing air to flow past the vocal membranes and subglottic pressure abruptly drops about 5 to 20 cm H_2O during the next 0.2 to 2.0 ms. In the case of single pulses this initial pressure drop is often followed by an increase of from 1 to 3 cm H_2O, after which subglottic pressure declines at a rate of about 0.5 to 2 cm H_2O/ms. When a group of pulses is emitted during a single respiratory cycle (Fig. 2, pulse train B), a large, rapid pressure drop is associated with the emission of each pulse within the group. This drop is followed within 3 to 5 ms by a return to about the pressure existing at the pulse onset (Fig. 2).

Subglottic Pressure and Sound Pressure Level

The peak sound pressure level of downward sweeping FM pulses emitted by these nine *Eptesicus* ranged from 93 to 118 dB (Table 1). Mean SPL averaged about 6 to 8 dB below peak values. The range of vocal intensity was comparable for single pulses and for pulses emitted in groups, whether spontaneous or induced by brain stimulation.

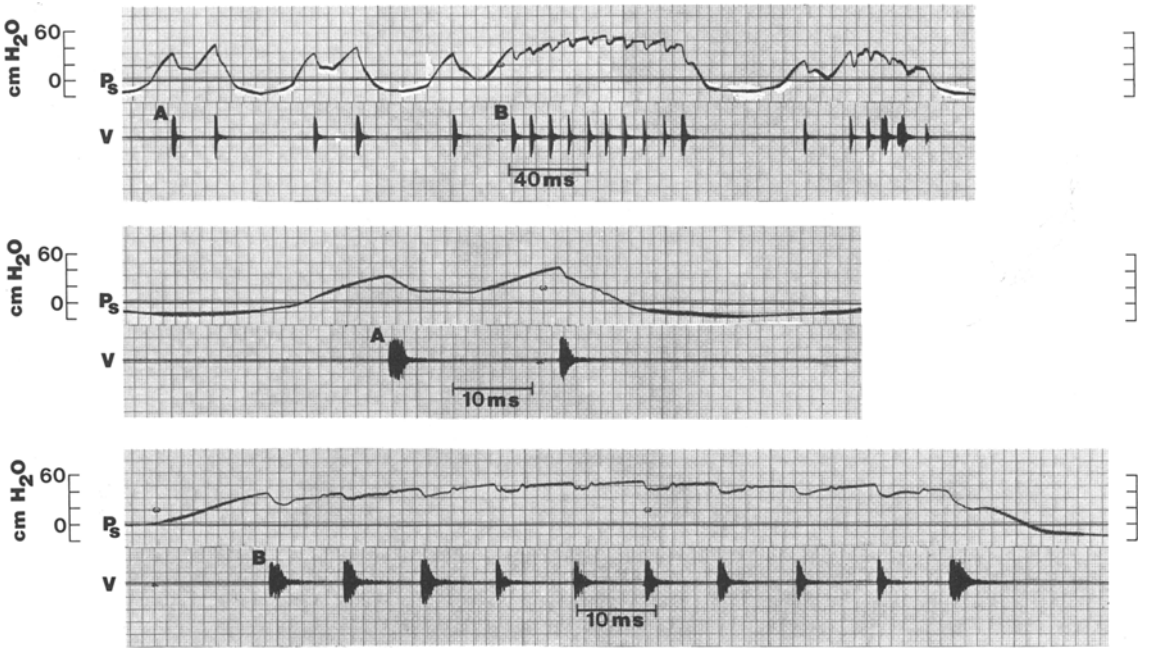

Fig. 2. Fluctuation in subglottic pressure (P_s) during the production of downward sweeping FM orientation pulses (V) at various pulse repetition rates by bat 8. Top tracings show two respiratory cycles during which a pair of echolocative pulses was emitted. These are followed by a single pulse and a 'buzz' containing 10 pulses in the next respiratory cycle. The time scale for pulse pair A and buzz B is expanded in the middle and lower tracings, respectively. The intermittent very high frequency, low amplitude sinusoidal oscillation on the middle and lower subglottic pressure traces is an electrical artifact and appears as a variation in the width of the line in the upper trace. Figure 2 shows sonagrams of pulses indicated by letters

Table 1. Subglottic pressure and sound pressure level

Bat no.	No. of vocalizations *n*	Sound pressure level[f] (dB re 2×10^{-5} N/m^2) Maximum	Mean	Subglottic pressure at pulse onset[f] (cm H_2O)	Exponent of subglottic pressure function[g] $SPL \propto P_s^x$
1[b, d]	46	105±4 (97–113)	97±3 (93–102)	37.7±15.1 (10–70)	0.73±0.08
2[a, e]	20	105±4 (93–112)	100±4 (90–106)	12.5±4.2 (6–24)	1.36±0.19
3[a, e]	30	110±4 (101–116)	104±4 (96–108)	43.4±8.8 (19–56)	1.01±0.30
4[b, e]	25	111±2 (106–114)	103±2 (99–106)	26.7±9.2 (14–50)	0.40±0.10
5[c, e]	27	110±3 (105–118)	104±2 (98–108)	27.4±6.7 (16–37)	0.90±0.14
6[b, d]	24	107±3 (101–111)	97±3 (92–101)	41.3±8.6 (28–55)	–
7[b, d]	49	109±4 (99–116)	99±3 (93–103)	32.5±12.7 (9–60)	0.79±0.10
8[b, d]	29	108±3 (103–114)	99±2 (95–104)	27.6±10.2 (13–47)	0.69±0.15
9[a, e]	38	111±4 (95–116)	107±4 (92–113)	33.7±12.2 (6–57)	0.91±0.05

[a] Single pulses
[b] Pulses in groups
[c] Single and groups
[d] Spontaneous
[e] Brain stimulation
[f] Mean ± standard deviation with range in parenthesis
[g] Exponent ± standard error

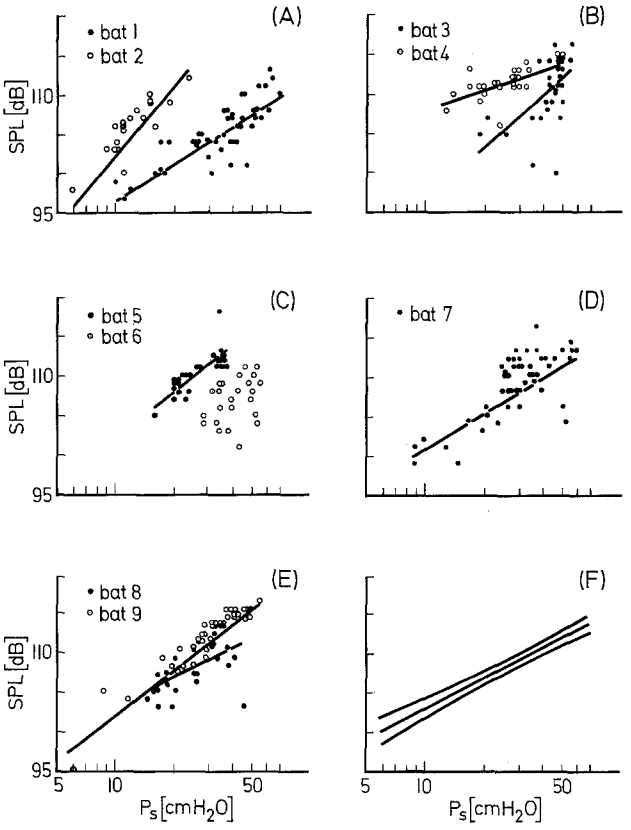

Fig. 3A–F. Maximum pulse sound pressure level as a function of subglottic pressure at pulse onset for nine individual bats (**A–E**), plus (**F**) regression line and 95% confidence limits for pooled data from eight bats (omitting bat 6). See Table 1

The maximum SPL of these ultrasonic echolocative pulses is significantly correlated ($P<0.001$) in eight out of nine bats with the magnitude, at pulse onset, of the subglottic pressure, which ranged from 6 to 70 cm H_2O (Table 1). The relationship between sound pressure level and subglottic pressure is shown graphically in Fig. 5.

The last column of Table 1 shows that the maximum SPL was directly proportional to the pulse onset subglottic pressure raised to a power ranging from 0.40 to 1.36 for the eight animals.

Although regression analysis failed to show a significant relationship between these variables in bat number 6, the slope of the regression line in the other eight animals ranged from 0.13 to 0.85 while the pooled data for these bats had a mean change in maximum SPL of 0.17±0.02 dB per cm H_2O change in subglottic pressure at the onset of vocalization ($P<0.001$). *Eptesicus* producing single pulses during a respiratory cycle (i.e., bats 2, 3, and 9 in Fig. 3) displayed regression functions with steeper slopes than did those animals emitting groups of pulses (i.e., bats 1, 4, 7, and 8 in Fig. 3). Bat 5 emitted both single pulses and groups of pulses.

No significant correlation between either inspiratory or expiratory volume and the SPL was evident. However, the whole-body plethysmograph may not accurately measure laryngeal airflow during intermittent phonation, due to the limited frequency response of the recording system and to the presence of distensible soft tissues, such as the cricothyroid membrane, below the glottis but outside the plethysmograph.

Subglottic Pressure and Pulse Frequency

Variation in the frequency of the emitted sound is often correlated with predictable changes in the subglottic pressure. Downward sweeping FM pulses (such as those in Fig. 1 and Fig. 2A, B, E, G) are accompanied by a decreasing subglottic pressure. However, constant frequency portions of pulses are typically accompanied by an almost constant sub-

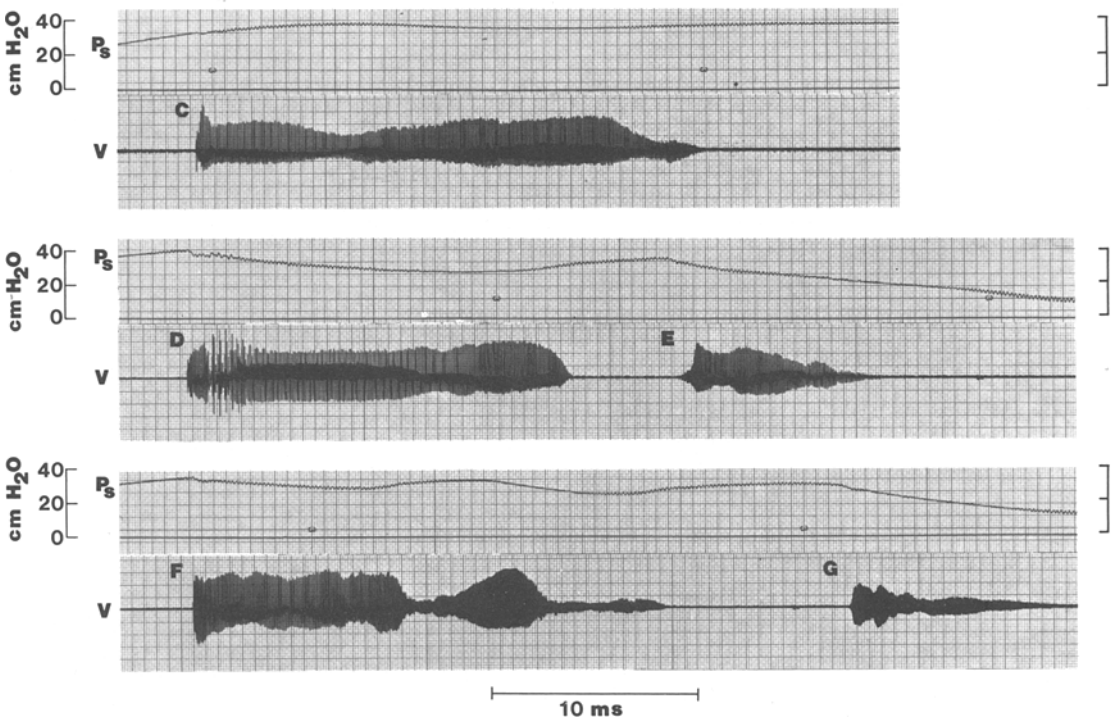

Fig. 4. Subglottic pressure (P_s) during the production of relatively long duration vocalizations (V) which contain ascending FM as well as nearly CF and descending FM. The sound spectrograph of each vocalization is shown in Fig. 2. Intermittent, low amplitude, high frequency sinusoidal oscillation in subglottic pressure is an electrical artifact

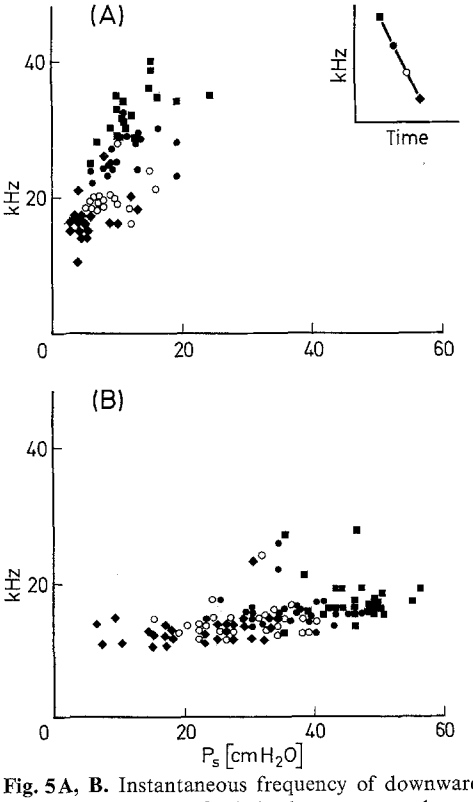

Fig. 5A, B. Instantaneous frequency of downward sweeping FM pulses as a function of subglottic pressure at the start of phonation and at the end of each quarter of the pulse. **A** and **B** are individual bats. Inset: points of measurement on a schematic sonagram of an FM pulse

glottic pressure. The same is true of ascending FM portions, except they are sometimes accompanied by a rising subglottic pressure (Figs. 1 and 4C, D, F). Sometimes phonation continues into the first part of the rising phase of subglottic pressure which precedes the next pulse. In such cases the pulse terminates with a brief constant frequency or rising FM tail. These roughly parallel changes in pulse frequency and subglottic pressure are especially noticeable in long duration vocalizations which include both rising and declining FM sequences (Fig. 4).

The relationship between frequency and subglottic pressure was quantified in two bats by measuring both of these parameters at the start of a number of downward sweeping FM pulses and at the end of their first, second, third and fourth quarter (Fig. 5). In both animals, the fundamental frequency declines as subglottic pressure decreases during the pulse ($P<0.05$), but the slope of the relationship between these parameters is much steeper in bat A than in bat B.

Data on pulse frequency and subglottic pressure are summarized in Table 2. Although the relative changes in subglottic pressures are correlated with the presence and direction of FM, Table 2 shows that the absolute value of the subglottic pressure at the start of an FM pulse is not always consistently related to the starting frequency of that pulse. The subglottic pressure at the beginning of phonation was

Table 2. Subglottic pressure and the regulation of pulse frequency and duration

Bat No.	No. of vocalizations	Fundamental frequency at pulse onset[f] (kHz)	Frequency sweep of fundamental[f] (octaves)	Absolute subglottic pressure drop during pulse[f] (cm H_2O)	Pulse onset frequency as function of onset P_s[g]	Duration[f] (ms)	Rate of subglottic pressure drop[f] (cm H_2O/ms)
1[b, d]	46	25±7 (15–36)	0.7±0.4 (0–1.6)	14.8±13.1 (3–58)	0.37±0.04 (0.85) $P<0.001$	4.6±1.8 (2–9)	3.4±3.2 (1–14.5)
2[a, e]	20	32±4 (25–40)	1.0±0.3 (0.4–1.5)	6.2±3.1 (2–14)	0.58±0.19 (0.59) $P<0.01$	9.7±4.8 (5–14)	0.6±0.2 (0.3–0.8)
3[a, e]	30	18±4 (15–29)	0.4±0.3 (0–1.0)	22.9±10.5 (5–41)	−0.12±0.08 (−0.27) $P<0.20$	6.9±2.9 (2–11)	3.7±1.9 (1.5–8.0)
4[b, e]	25	24±2 (20–28)	0.9±0.2 (0.6–1.3)	16.4±7.8 (4–30)	0.10±0.05 (0.40) $P<0.05$	6.0±1.5 (3–9)	2.6±1.0 (1–5)
5[c, e]	27	28±4 (17–33)	0.6±0.3 (0.2–1.2)	14.3±5.4 (7–25)	−0.20±0.11 (−0.34) $P<0.10$	33.0±18.1 (5–73)	0.5±0.3 (0.3–1.5)
6[b, d]	24	33±6 (25–48)	1.0±0.2 (0.5–1.4)	12.6±10.3 (1–38)	0.15±0.15 (0.21) $P<0.40$	3.3±1.5 (1–6)	3.9±3.7 (0.5–19.0)
7[b, d]	49	23±5 (16–48)	0.9±0.3 (0.3–1.4)	22.2±10.2 (5–46)	0.07±0.06 (0.17) $P<0.30$	3.5±1.1 (1–5)	6.5±3.2 (1.7–15.3)
8[b, d]	29	29±6 (18–44)	0.7±0.3 (0.1–1.8)	12.6±7.5 (2–29)	0.05±0.11 (0.08) NS	2.4±0.8 (1–5)	5.0±2.8 (2.0–14.5)
9[a, e]	38	28±7 (12–41)	0.6±0.3 (0–1.1)	26.8±11.9 (1–48)	0.27±0.09 (0.45) $P<0.01$	11.4±3.2 (2–17)	2.5±1.2 (0.2–5.1)

[a] Single pulses
[b] Pulses in groups
[c] Single and groups
[d] Spontaneous
[e] Brain stimulation
[f] Mean ± standard deviation with range in parentheses
[g] Regression slope ± standard error with correlation coefficient in parentheses

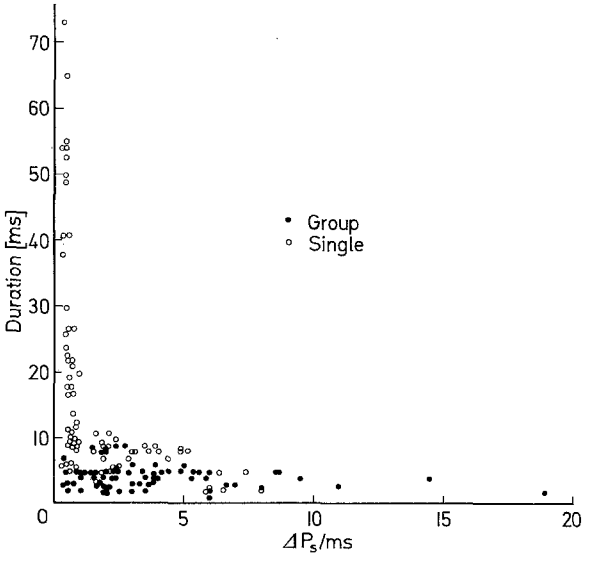

Fig. 6. FM pulse duration as a function of the rate of drop in subglottic pressure during the pulse. Multiple pulses per respiratory cycle indicated by solid dots, single pulses indicated by open circles. See also Table 2

significantly correlated with the initial frequency of downward sweeping FM pulses in only four out of nine bats studied.

Effect of Pulse Duration on Subglottic Pressure and Tidal Volume

The rate of drop in subglottic pressure is plotted in Fig. 6 as a function of pulse duration. The mean values of these quantities for each bat is given in Table 2. Although the greatest rates of subglottic pressure drop are associated with short pulses having durations from less than 5 to 10 ms, the precise relationship between these variables and its significance is uncertain. Pulses emitted after brain stimulation averaged about two to four times longer than those produced spontaneously (Table 2), but this may be largely due to the fact that most of the former were single pulses, whereas many spontaneous pulses occurred in groups. The whole-body plethysmograph makes it possible to measure changes in lung volume during phonation without obstructing the mouth or nostrils. These data show that *Eptesicus* increases its tidal volume during phonation. Resting tidal volumes ranged between 0.05 and 0.1 ml in silent bats weighing between 18 and 26 g. Long duration pulses were accompanied by large tidal volumes up to about 0.4 ml, compared to tidal volumes of as little as 0.1 ml for single short pulses.

Lung Compliance

In order to assess the possible effect of lung compliance on subglottic pressure, the static lung compliance was determined in isolated lung preparations of eight *Eptesicus*. The mean static lung compliance of these animals was 0.021 ± 0.002 ml/cm H_2O and the mean specific lung compliance was 1.3 ± 0.2 ml/cm H_2O/kg body weight, suggesting a relatively stiff lung.

Discussion

The Generation of Echolocative Pulses

The larynx of *Eptesicus* has several anatomical specializations for the production of ultrasound that are not present in other mammals. One of the most important of these is the presence of thin membranes on the vocal folds (and to a lesser extent on the ventricular folds) which, because of their low mass, can vibrate at ultrasonic frequencies and generate the laryngeal waveform (Griffin 1958; Novick and Griffin 1961; Suthers and Fattu 1973). It seems unlikely that these delicate structures provide the glottal resistance needed to develop the high subglottic pressures associated with phonation. By determining airway pressures at various points along the vocal tract, Suthers and Fattu (1973) demonstrated that the glottal stop involved in the production of echolocative pulses lies between the posterior pharynx and upper trachea. They postulated that there is a separation of vibratory and resistive structures in the larynx of echolocating bats and proposed that the glottis consists of a muscular hump forming the base of the vocal fold. Unlike the rest of the laryngeal cavity, which is lined with pseudostratified ciliated columnar epithelium, this muscular hump is lined with stratified squamous epithelium.

We believe these folds are adducted prior to phonation, closing the glottis and allowing expiratory effort to build up subglottic pressure. Phonation is assumed to begin when subglottal air pressure becomes great enough to overcome the glottal adducting forces. At this point the glottis opens slightly causing an initial abrupt pressure drop. Air rushing through the glottis creates Bernoulli or other aerodynamic forces which combine with the elastic recoil and tension in the vocal membranes, drawing them medially and setting them in oscillation. The frequency of the waveform generated within the larynx is determined primarily by the tension exerted by the cricothyroid muscles on the vocal membranes, while its intensity depends primarily on the amplitude of membrane vibration.

All of the vocalizations we have studied, with the exception of a few broad-band click-like sounds, are produced during expiratory airflow when there is a positive subglottic pressure and when plethysmographic records show expiratory maneuvers. This conclusion is supported in *E. fuscus* by airflow measurements based on the output of a small thermistor in front of the mouth (Suthers and Fattu 1973). Similar findings have been reported for the echolocating bats *Phyllostomus hastatus, Eptesicus serotinus* and *Myotis myotis*, by previous investigators using various techniques (Schnitzler 1968; Roberts 1972b; Suthers et al. 1972). Groups of pulses which may extend into the inspiratory phase of respiration are also associated with brief expiratory maneuvers (Suthers and Fattu 1973).

Plethysmographic data show that *Eptesicus* increases its tidal volume during phonation. A similar phenomenon is well known in man (Draper et al. 1959; Bouhuys et al. 1966), but comparative data during phonation by other small mammals are not available.

At low repetition rates a single pulse may be emitted during a respiratory cycle. *Eptesicus* starts these pulses at a high subglottic pressure and thus achieves a high SPL. By restricting the rate of pressure drop to about 0.2 to 2 cm H_2O/ms, subglottal air is conserved allowing a longer duration vocalization to be attained. Extremely long vocalizations, such as those lasting from 20 to 70 ms, appear to attain a minimum rate of pressure drop of about 0.2 cm H_2O/ms which may represent the maximal glottal resistance at which phonation can be achieved. Sustained high glottal resistance also favors prolonged high intensity. Increased energy expenditure by expiratory muscles in maintaining airflow against the high glottal resistance may be necessary, however. Slower rates of frequency modulation during these pulses may represent some degree of interdependence between the muscles regulating vocal membrane tension and those controlling glottal resistance.

At high repetition rates, groups of short duration pulses having high rates of frequency sweep are emitted during a single respiratory cycle. Such pulses do not allow time for slowly building up subglottic pressure over the 15 to 50 ms before phonation as in the case of single pulses. *Eptesicus* overcomes this problem at high repetition rates by sustaining a high DC level of subglottic pressure which is rapidly released and reset with each pulse. Although high rates of subglottic pressure drop are attained, e.g., 5 to 13 cm H_2O/ms, the brevity of each pulse apparently does not deplete the subglottic air supply. A group of pulses may occupy only 5 to 15% of the total expiratory time in comparison to from 4 to 50% occupied by a single pulse.

Subglottic Pressure and the Regulation of Sound Pressure Level

When considering the relationship between subglottic pressure and the SPL, it is important to evaluate the extent to which variation in the recorded sound pressure level actually represents changes generated at the animal's mouth. The SPL of vocalizations is affected by several factors including atmospheric attenuation, spreading losses and airway damping. Atmospheric attenuation calculated for undried air (RH 37% at 26.5 °C) accounts for approximately 0.03 dB drop at 10 kHz and 0.3 dB at 50 kHz over the 15 cm from bat to microphone (Beranek 1967). This loss is constant during an experiment and hence does not affect interpretation of the data.

Spreading losses associated with the pattern of sound radiation from the mouth influence the SPL measured at any particular point in front of the animal (Schnitzler 1968; Simmons 1969; Griffin and Hollander 1973; Shimozawa et al. 1974). Bats 1 to 8 were immobilized such that no head movement was possible. Bat 9 was limited to a maximum head movement of +5 degrees in the plethysmograph. It is therefore assumed that spreading losses varied relatively little under the conditions of these experiments.

Airway damping due to the T-cannula coupling the pressure transducer was estimated to cause a 3 to 8 dB reduction in the SPL of the emitted sound (see Materials and Methods). This important effect was likewise constant for a given experiment.

The physiological control of the intensity of the human voice is complex. Among other things, it includes glottal airflow and resistance, supraglottal resonances and mouth radiation. The driving force of vocal intensity, the subglottic power, is the product of subglottic pressure (P_s) and airflow (F). Since P_s is linearly proportional to F, one might expect the vocal intensity to be roughly proportional to P_s^2 and that the SPL should therefore be proportional to P_s (van den Berg 1956). The approximately linear relationship which we observed in most of our *Eptesicus* (we have no explanation for the absence of a significant correlation in bat 6) is thus in fairly good agreement with the predicted relationship between these variables.

In man, however, SPL has been found experimentally to be proportional to P_s^2 (van den Berg 1956), $P_s^{1.6}$ (Ladefoged and McKinney 1963) or $P_s^{1.65}$ (Isshiki 1964). In dogs, Koyama et al. (1969) found an exponent of 2.15. Van den Berg (1956) ascribed the unexpectedly high exponent which he observed in man to the effect of vocal tract resonances and to the fact that in man the area of the radiating mouth increases with sound level. Changes in vocal tract shape also have an important effect on the intensity of human speech (Stevens and House 1961).

There is some evidence that *Eptesicus* does not modulate the SPL of its echolocative pulses by altering mouth radiation or vocal tract tuning. If van den Berg's hypothesis is correct this difference from humans may explain the linear, rather than quadratic, relationship between P_s and SPL in this bat. Shimozawa et al. (1974) found that sound emission in the closely related vespertilionid, *Myotis*, occurred only during the maximal opening of the mouth, suggesting the aperture varies little during phonation. Studies by Roberts (1973) of bats vocalizing while breathing light gas (HeO_2) mixtures show that the harmonic spectra of FM bats, such as *Eptesicus serotinus*, are not shifted under such conditions. FM bats thus appear to have a broadly tuned vocal tract. The vocal intensity of *Eptesicus* is therefore probably controlled by glottal and subglottal regulatory mechanisms. This may not be true of constant frequency bats, for which light gas experiments suggest a sharply tuned vocal tract. Exponents of less than one which were observed in some *Eptesicus* may be due to glottal attenuation, energy loss in laryngeal vibration and unmodulated expired air (Isshiki 1964).

Alternatively, the quadratic relationship between SPL and P_s found by others in humans and dogs may not depend on properties of the vocal tract or changes in mouth radiation as von den Berg (1956) suggested. Cavagna and Margaria (1965) found that a similar relationship was exhibited by mechanical models in which the effect of the vocal tract is ruled out. They suggest that the non-linear relationship between these variables in man and dogs must be an intrinsic property of the sound generator itself. If so, perhaps the unique, thin vibratory membranes on the edges of the vocal folds in echolocating bats behave differently from the vocal folds in other mammals and are at least partly responsible for the linear relationship we observed in *Eptesicus*.

Ultrasonic vocalizations produced by *Eptesicus* are of high intensity compared to most biologically produced sound. Our data strongly suggest that this is achieved, to an important extent, by the development of exceptionally high subglottic pressures which presumably cause the laryngeal membranes to vibrate with a greater amplitude. Subglottic pressure in man, for example, typically reaches only about 8 cm H_2O during normal speech (Ladefoged 1968) and 20 to 30 cm H_2O when shouting (Isshiki 1964).

One wonders what special adaptation *Eptesicus* might have developed in order to tolerate such pressures. The measurement of the static lung compliance of eight bats (Table 3) indicates that the bat lung is stiffer than that of many other mammals. Its low

Table 3. Static lung compliance in various mammals

Species	Static lung compliance		Body wt kg	*n*	Reference
	Absolute ml/cm H_2O	Specific ml/cm H_2O/kg B.W.			
Bat	0.021	1.28	0.016	8	Present Study
Mouse	0.049	1.53	0.032	4	Crosfill and Widdicombe (1961)
Guinea pig	0.20	0.91	0.219	200	Amdur and Mead (1958)
Rabbit	6.0	2.50	2.4	4	Crosfill and Widdicombe (1961)
Man	0.048	2.86	70	–	Altman and Dittmer (1971)

compliance may increase its recoil pressure and thus increase the driving force for phonation. A non-compliant lung also may prevent early airway closure at high positive intrathoracic pressures characteristic of phonation. In addition, the ventral surface of the larynx is covered by a large elastic cricothyroid membrane (Elias 1907). This membrane could potentially act as a compliant reservoir of subglottic air that is filled immediately prior to phonation and provides a larger volume of air at a high subglottic pressure during phonation. All of these adaptations would be of benefit to the bat in permitting the development of higher intensity orientation pulses and therefore increasing the range of echolocation and improving the detection of small objects.

Subglottic Pressure and the Regulation of Frequency

Major structural differences between the microchiropteran and other mammalian larynges make comparison of frequency regulatory mechanisms hazardous. The vocal folds of man are relatively large, bulky structures which can be intrinsically regulated by the vocalis muscle. They thus contrast with the thin, amuscular membranes in the microchiropteran larynx which are believed to be the vibratory source of ultrasounds (Elias 1907; Griffin 1958; Novick and Griffin 1961; Suthers and Fattu 1973). Regulation of length and tension in these thin homogeneous membranes can only be by muscles acting across them.

Ultrasonic pulses emitted by *E. fuscus* in these experiments tended to be lower in frequency than those reported during free ranging, insect pursuit and orientation tasks by intact bats (Griffin 1958, Simmons and Vernon 1971). Surgical cannulation and coupling to the transducer probe may be largely responsible for this difference. Laryngeal muscles may also have been operating at temperatures slightly lower than normal. Lowered pulse frequency is not due to brain stimulation since, in cannulated bats, the onset frequency of pulses elicited by brain stimulation was comparable to that of spontaneously produced pulses (Table 2).

Physiological evidence suggests that the primary determinant of pulse frequency in vespertilionid bats is the tension applied to the laryngeal membranes by the cricothyroid muscles. Visual observation of fresh dissected larynges has demonstrated that the laryngeal membranes are tensed after cricothyroid muscle stimulation (Griffin 1958). Denervation of the cricothyroid muscle drastically reduces pulse frequency into the audible range and eliminates frequency modulation (Griffin 1958; Novick and Griffin 1961; Suthers and Fattu, submitted). Electrical activity in the superior laryngeal nerves and within the major ventrolateral portion of the cricothyroid muscle precedes pulse production causing muscle tension to peak just prior to phonation with a decrease in tension during phonation due to gradual relaxation (Suthers and Fattu 1973).

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