

ULTRASONIC VOCALIZATIONS OF FLYING BATS MONITORED BY RADIOTELEMETRY

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Summary

Ultrasonic vocalizations of flying bats were effectively monitored with radiotelemetry. We describe a device light enough to be carried by an 11g bat for periods of up to 1h. It transmitted signals adequate for fine frequency analysis within a range of approximately 3 m. Telemetry permitted the recording of constant-frequency pulses free from flight-induced Doppler shifts and without time delays. The difference in frequency between telemetered signals and the same signals detected by a remote microphone was used to calculate velocity and Doppler shifts.

Pulse emission behavior of *Pteronotus parnellii* in flight was compared with simulated flight on a pendulum. The data showed significant differences in echo bandwidths, constant-frequency pulse durations and interpulse intervals. In flight, pulses and interpulse intervals tended to be shorter and bats maintained echo frequencies within a significantly narrower band. Phases of echolocation that characterized the approach to a target were clearly evident in flight, but not during pendulum swings. Differences in pulse durations and interpulse intervals may be correlated with the integration of wingbeat, respiration and vocalization. The absence of wing motion in simulated flight changes this integration.

Introduction

Recordings of vocalizations in flying bats led to the discovery of echolocation. Early studies described interspecific variation in the structure and timing of the vocalizations and systematic phases of echolocation (search, approach and terminal) as bats foraged for prey (see Griffin, 1958; Griffin *et al.* 1960). Most species were found to emit broad-band, frequency-modulated (FM) pulses, while some used pulses with a constant-frequency (CF) component terminated by an FM sweep. Bats which employed the CF–FM pulses, *Pteronotus parnellii* and members of the families Rhinolophidae and Hipposideridae, precisely altered the CF component of their pulses in order to compensate for Doppler shifts induced by flight velocity. This compensation functioned to keep returning echoes within a preferred band (reference frequency) to which the ears are sharply tuned (see Schnitzler and Henson, 1980).

Key words: bats, *Pteronotus parnellii*, echolocation, radiotelemetry.

Studies of Doppler-shift compensation in flying bats required the determination of pulse frequency without errors induced by the Doppler effect. This led to the development of techniques either to eliminate the error or to correct for it. Various methods have been employed, such as high-speed photography to determine flight velocity (Schnitzler, 1970), wind-tunnel flights to enforce a constant velocity (Schnitzler, 1973), electronic simulations to control the frequency of Doppler-shifted echoes presented to a stationary bat (Schuller *et al.* 1974) and swinging a target towards a stationary bat to produce known patterns of Doppler shift (Schnitzler, 1968). In a variation on the latter technique, Henson *et al.* (1982) placed a bat on a pendulum and swung it towards a target. With a microphone mounted on the pendulum, vocalizations could be recorded free from Doppler shifts, and the Doppler-shifted echoes could be recorded simultaneously as the bat would hear them. This method of flight simulation has been used to evaluate Doppler-shift compensation in several bats (Henson *et al.* 1982; Habersetzer *et al.* 1984; Kobler *et al.* 1985).

To record the emitted pulses in free-flying bats, Henson *et al.* (1987) attached a microphone to a miniature FM radio transmitter. This system telemetered vocalizations without Doppler-induced error. While initial results were promising, the transmitter's weight impaired flight, and nulls in reception resulted in sporadic interruption of the signal.

We have experimented with other transmitter designs using new, light-weight components and an array of antennas which minimize reception nulls. In this study we examine the methodology and utility of vocalization radiotelemetry and aspects of echolocation behavior that cannot be addressed with other methods. We also compare the pulse emissions of flying bats with sounds emitted by the same bats on a pendulum.

Materials and methods

Four *Pteronotus parnellii parnellii* captured in Jamaica and maintained in captivity on a diet of mealworms (*Tenebrio* larvae) and vitamin supplements were used in the experiments. Animals were housed in an enclosure large enough (300cm × 210cm × 170cm) to allow flight and were exercised daily in a large room. Body masses ranged from 11 to 12g.

A miniature FM transmitter was used to record the emitted pulses from flying bats. The transmitter was constructed of surface-mount electronic components with a design similar to that of Skutt *et al.* (1967) (Figs 1 and 2A). An electret microphone (Knowles, model 1759 with adequate sensitivity to 75kHz) was coupled to the transmitter, which consisted of two NPN transistors, a three-turn, hand-wound induction coil (22 gauge copper wire) and eight other passive components. The transmitter complete with 1.5 V silver oxide battery weighed 0.85g. The entire assembly was attached to a thin, contoured plastic cap which roughly matched the dorsal surface of the bat's head.

Precise tuning of the transmitter was critical and was accomplished by compressing or expanding the induction coil to produce a carrier frequency within the 88–108MHz FM band. Selecting a band free from commercial broadcast interference was important and

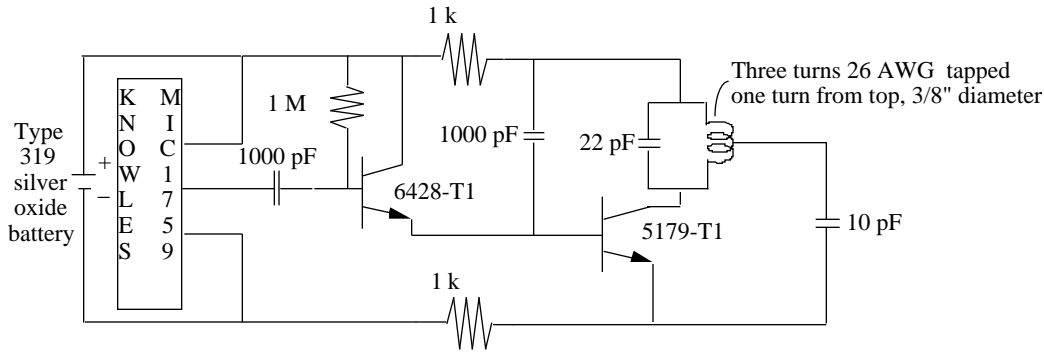


Fig. 1. Schematic diagram of the FM transmitter.

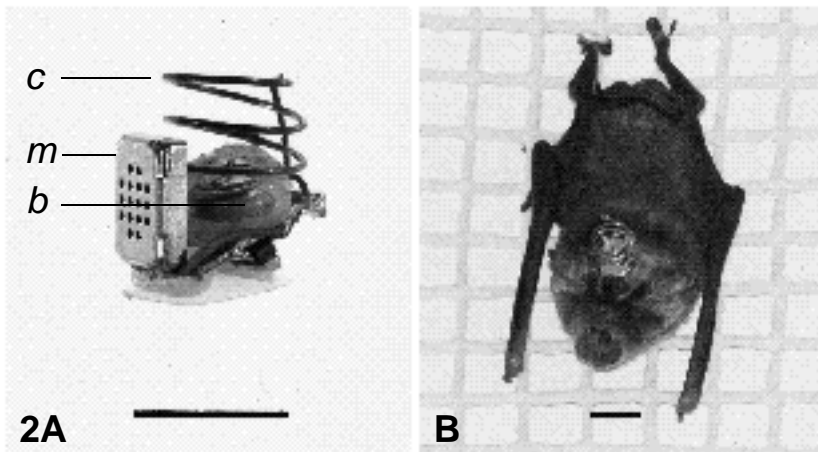


Fig. 2. Photograph of the FM transmitter (A). The electret microphone (*m*) faces anteriorly. The three-turn copper coil (*c*) serves as the transmission antenna and is positioned over the battery (*b*). Photograph of bat with the transmitter attached to its head (B). Scale bars, 1cm.

difficult to achieve. When properly tuned, the transmitter provided an adequate signal, as long as the bat remained within the effective range.

To attach the transmitter, the bat's head was tonsured with depilatory cream (Neet) and both the skin and plastic cap were thinly coated with cosmetic latex (Lashgrip eyelash adhesive). The latex was allowed to dry before the device was pressed into place. This simple procedure provided secure attachment (Fig. 2B). The procedure was performed on conscious animals and did not appear to cause overt distress. Animals seemed to acclimate to the presence of the transmitter and did not make obvious attempts to dislodge it. At the end of the experiment, the transmitter could be easily removed and there was no discernible irritation to the skin.

Bats used in these experiments were able to carry the transmitter without apparent impairment for periods of an hour or more. After that, some showed signs of fatigue, as shown by poor maneuverability or frequent landing. After this had been established,

animals were allowed to rest at will, and recording sessions seldom extended beyond 45min.

Flights were made under two sets of conditions. In the first experiments with one bat, flights were in a floor-to-ceiling mesh enclosure (80cm × 245cm × 245cm). A piece of poster board at one end served as a target. A variable-position port (quarter-round opening measuring 37cm in radius) in the target provided the opportunity to escape from the small enclosure into a larger room and gave the bat an incentive to echolocate the target. Changing the port's position after each escape required the animal to negotiate a different path during each trial. A stationary microphone (6.25mm Brüel and Kjær) at the escape port recorded emissions from the approaching bat. After an escape, the bat was allowed to fly or rest briefly (at its discretion) prior to the next trial.

In a second set of experiments, four bats were used. Flights were conducted in a 650cm × 150cm section of a concrete-walled corridor, enclosed at each end with a fabric net. This chamber had limited places to land which encouraged landing at a target where a microphone was positioned. The bats were allowed to land only at or near the target and were discouraged from landing away from the target by immediately startling them into flight again. Flight in this enclosure was less constrained than in the small chamber. Brief rest periods were allowed after each appropriate landing.

Telemetered signals were received by a pair of folded-dipole antennas (300 antenna wire) the dimensions of which were set to match the carrier frequency of the transmitter. These antennas were spaced approximately three-quarters of a wavelength (250cm) apart and oriented to optimize reception. Reception was best in the near field when the animal flew past the antenna array. Signal strength diminished when the bat moved away from the array, and was inadequate at ranges beyond approximately 3m. Use of an FM amplifier (Archer model 15-1105) in the antenna cable significantly improved signal-to-noise ratio. Nulls, which proved to be a significant hindrance, were overcome by manipulating the antenna array. This was a process of trial and error which varied among different recording sites.

The transmitter output was received with a commercial FM tuner (Harmon and Kardon, model T403), equipped so that high-frequency signals could be recorded from a discriminator. Voiced comments during flights, also telemetered by the bat's transmitter, were recorded from the audio output of the tuner and facilitated location of good taped sequences.

All signals were recorded on a Racal Instrumentation (Store 7 DS) tape recorder. Both the telemetered and stationary microphone signals were bandpass-filtered to isolate the second harmonic (approximately 61kHz) constant-frequency component of echolocation calls. The difference in frequency between the transmitted vocalization and the signal recorded by the stationary microphone was used to calculate instantaneous velocities and Doppler shifts by the formulae:

$$v_b = - \left(\frac{f_p c}{f_t} \right) + c, \quad (1)$$

$$f_e = f_t \left(\frac{c + v_b}{c - v_b} \right), \quad (2)$$

where f_e is the CF echo frequency, f_p is the CF pulse frequency recorded by telemetry, f_t is the CF pulse frequency recorded at the stationary target microphone, v_b is the flight velocity of the bat and c is the velocity of sound, 344mmms^{-1} . Frequency and pulse-echo timing were measured on a Rapid Systems R350 FFT spectrum analyzer/digital oscilloscope. This system provided a resolution of $\pm 15.6\text{Hz}$. Pulse durations and interpulse intervals were determined by measuring the onsets and terminations of the telemetered signals as portrayed by the digital oscilloscope. Data included only those portions of the initial and terminal FM pulses that fell within the limits of roll-off of the filter (-3 dB at 59.7kHz and 65.0kHz , $f_{\text{max}}=62.5\text{kHz}$) at a sound pressure level sufficient to be visible on the oscillograph. Oscillographs were printed on an electronic chart recorder (Gould ES 1000). Data analysis and storage were executed with Microsoft Excel.

Data obtained during flights in the large chamber were compared with those collected during simulated flight on a pendulum using the same bats. (Comparative pendulum data were not collected for flights in the small chamber.) The pendulum system used was similar to that of Henson *et al.* (1982). Only data from forward swings were analyzed. We compared three variables: CF pulse duration, interpulse interval and echo frequency bandwidth. Mean durations and mean echo frequencies were used to analyze differences between flights and pendulum swings with a *t*-test and *F*-test, respectively. Interpulse intervals showed a positively skewed distribution. Therefore, we tested for differences between long and short intervals using the Cochran test, developed for comparing means of independent populations having unequal variances (Snedecor and Cochran, 1980).

Results

Telemetered vocalizations combined with recordings from stationary microphones provided signals adequate for the analysis of Doppler-shift compensation and flight velocity. Timing patterns of pulse emission could also be extracted from telemetered signals. Fig. 3 shows oscillographic traces of vocalizations monitored simultaneously by telemetry and using a stationary microphone. Although the signal-to-noise ratio from the stationary microphone was clearly better than that from the transmitter (while the animal flew towards the microphone), telemetered signals faithfully represented the animal's pulse emissions.

Velocity

In flight, maximum velocity was 4.66ms^{-1} and the average velocity in search phase was 3.00ms^{-1} (± 0.691 , $N=372$). Velocity typically decreased during the approach phase. Velocity of a pendulum is determined by the acceleration due to gravity and the maximum depends on the length of the arm of the pendulum and the distance of the swing. Velocity of the pendulum used ranged from 0 to 3.6ms^{-1} .

Doppler-shift compensation

Under all experimental conditions, *P. parnellii* Doppler-shift compensated. Table 1 lists the mean frequency and standard error of the mean of pulses and echoes during

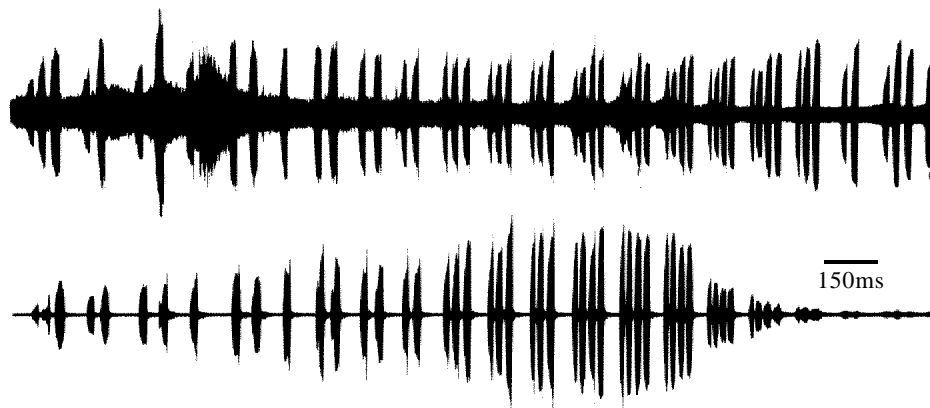


Fig. 3. Oscillograph of vocalizations from a flying *Pteronotus parnellii* as monitored simultaneously by telemetry (above) and a stationary microphone (below). Except for brief periods of reduced signal-to-noise ratio, the record shows that the telemetered signal maintained a relatively constant amplitude as the bat approached and turned away from the target with a stationary microphone. By contrast, the signal from the stationary microphone changed with respect to the bat's position.

Table 1. Summary of data for flights and pendulum swings

Bat	Mean pulse frequency (Hz)	Mean echo frequency (Hz)	No. of flights or swings	Total no. of pulses	Mean frequency range (Hz)	
					Pulses	Echoes
Flight data						
NB	61743 (78.2)	62556 (21.5)	2	52	1594	828
WL	60837 (22.9)	61826 (10.5)	7	183	1359	1219
WR	60285 (16.1)	61235 (12.9)	5	167	1016	1234
YR	60242 (16.0)	60937 (7.9)	8	239	1188	531
Pendulum data						
NB	61854 (29.8)	62608 (26.3)	5	102	1328	1406
WL	60996 (30.4)	61658 (28.9)	4	93	1281	1094
WR	60670 (28.0)	61345 (25.4)	4	115	1078	1313
YR	60228 (36.9)	60844 (27.3)	4	75	1172	1219

Values in parentheses are standard errors.

flights and pendulum swings. In flights and swings, the standard error of echoes is less than that of pulses. The bats maintained echo frequency in a narrower band than pulse frequency, clear evidence of Doppler-shift compensation. The accuracy of compensation

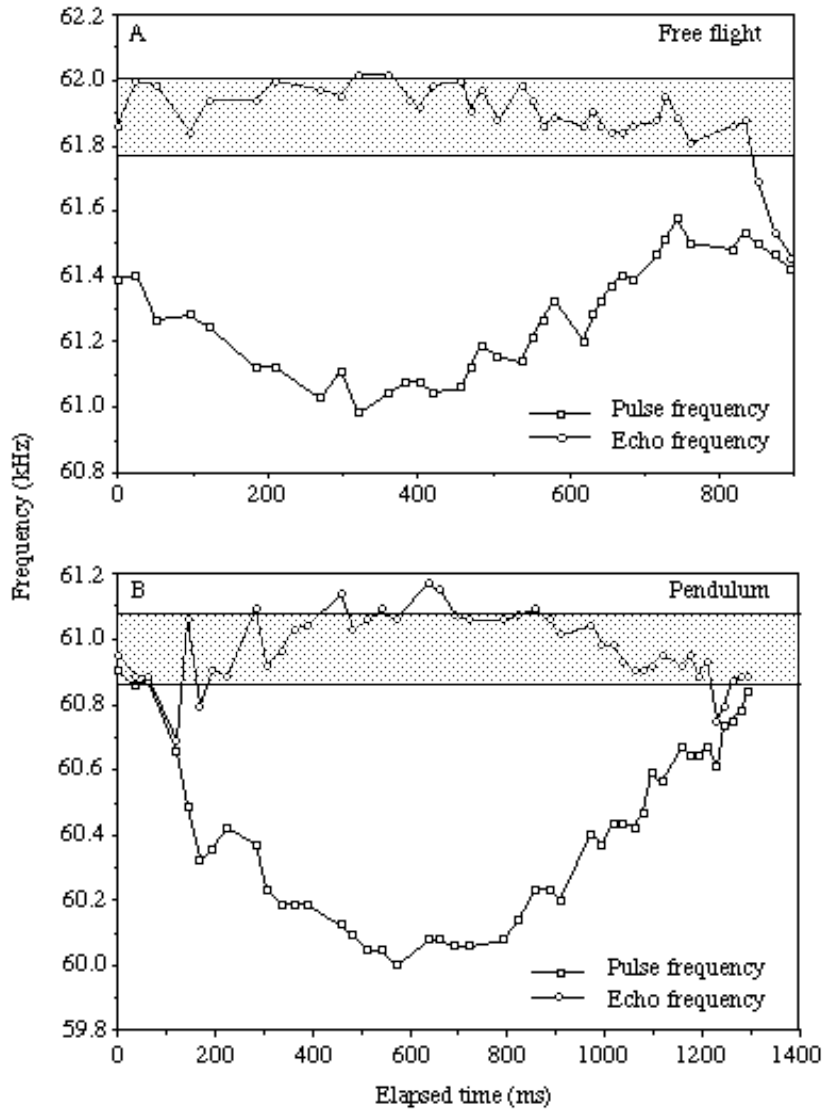


Fig. 4. Frequency vs time plot of emitted pulses and echoes of a bat in flight (A) and on the pendulum (B). In both cases, the shaded bar overlying the echo trace represents one standard deviation about the mean frequency of the echoes in that trial. In both cases, the echo trace falls out of the shaded band at the end of the sequence as the bat's velocity decreased during the approach to a target. The animal had ceased to Doppler-shift compensate accurately.

was not consistent throughout flights and pendulum swings. Bats which compensated well in the course of a flight would often cease to compensate immediately before landing, turning, flying through the escape port or at the end of the pendulum swing. Echo frequencies were restricted to a narrow band during most of the flight or swing but dropped echoes out of that band prior to the end of the vocalization sequence (Fig. 4A,B). This was seen in 19 of 27 flights (5 in the short chamber, 22 in the long chamber) and 10 of 17 pendulum swings.

Table 2. *F-test of echo frequency data*

Bat		Mean echo frequency (Hz)	Sample variance	<i>F</i>	d.f.
NB	Flight	62556	23992.3	2.9295	51 *
	Pendulum	62608	70284.4		101
WL	Flight	61826	19620.5	3.9643	200 *
	Pendulum	61658	77782.1		92
WR	Flight	61235	27439.2	2.6932	166 *
	Pendulum	61345	73898.7		114
YR	Flight	60937	14745.9	3.7789	238 *
	Pendulum	60844	55723.3		74

* Significant difference, $P < 0.01$ between the values for flight and pendulum.

Echo frequencies

Bats in flight maintained echoes near their reference frequency with high precision. Table 2 lists the mean echo frequencies of flights and pendulum swings for each bat separately. For echoes, flight and pendulum data for each bat have significantly different variances based on the *F*-test ($P < 0.01$). Thus, in all cases we have rejected the null hypothesis that the two samples come from one population with a single variance. Fig. 5A,B shows histograms of echo frequencies (in 50Hz bins) for all flights and pendulum swings for all four bats. Echo frequencies for each animal are normalized to the bin which contained the greatest number of observations. Considered as the reference frequency, this bin was set equal to zero so that data from all animals could be pooled. Echoes above and below the reference frequency were labelled positive and negative, respectively. 26% of all echoes recorded in flight fell into the reference frequency bin; 59% were within three bins centered on the reference frequency (range 150Hz). On the pendulum, the modal bin comprised only 11% of values. A range of 300Hz about the modal value contained 47% of all echoes.

Pulse duration

Duration of the constant-frequency portion of pulses in free flight (Fig. 6A) ranged from 4 to 23ms. Durations between 10 and 18ms occurred most frequently. For all flights, the mean pulse duration was 13.2ms. Constant-frequency pulse durations on the pendulum ranged from 9 to 25ms; most were between 12 and 20ms (Fig. 6B). Overall, the mean pulse duration on the pendulum was 16.8ms. For each bat, mean pulse durations in flight were significantly shorter than mean pulse durations on the pendulum ($P < 0.01$).

Interpulse interval

Interpulse intervals separated into two groups. Long intervals occurred between groups of two or more pulses, while short intervals came between successive pulses in a group. Fig. 7A depicts interpulse interval duration as a function of time in a flight. During search-phase emissions, trains of two pulses were represented by alternating long and

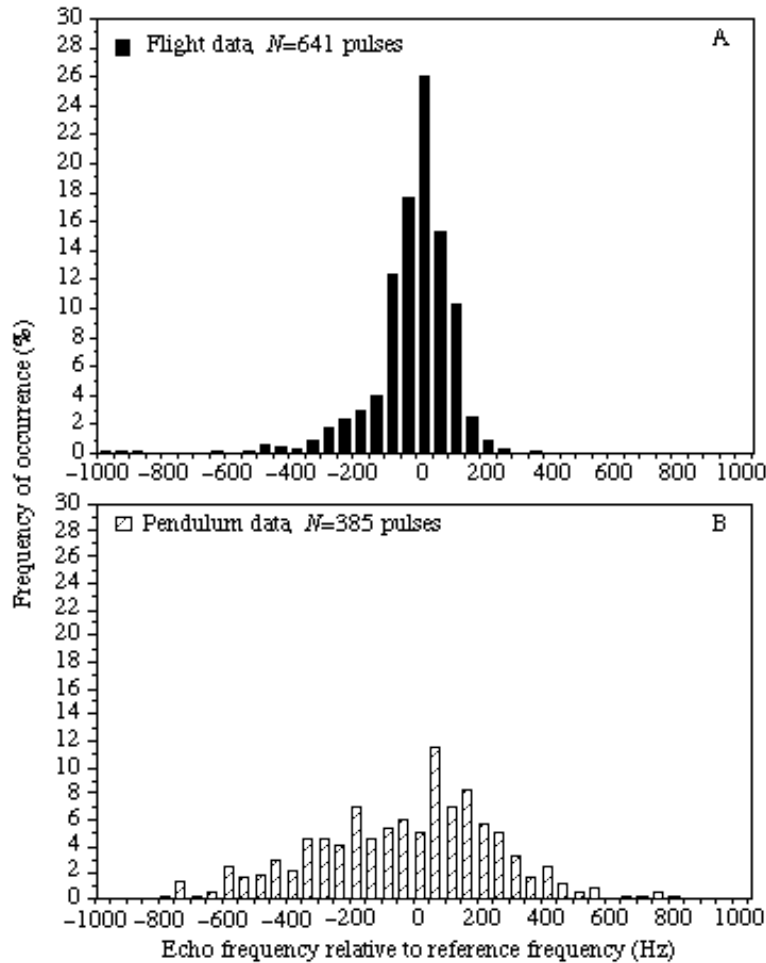


Fig. 5. Histogram of echo frequencies of all four bats in all flights (A) and pendulum swings (B) standardized to reference frequency (set to zero). Note that echo frequency is maintained in a narrower band in flight than on the pendulum.

short intervals. As the repetition rate increased, multiple short intervals were interspersed between long intervals. Both short and long intervals progressively shortened and repetition rate increased as the animal approached a landing area. Interpulse intervals on the pendulum (Fig. 7B) did not show the same pattern. High repetition rates often occurred early in the swing, and sometimes abruptly decreased.

Interpulse interval groups are depicted in histograms for each flight or swing in one bat in Fig. 8A,B. In all flights, mean short intervals ranged from 5 to 19ms and mean long intervals ranged from 25 to 84ms. Over all pendulum swings, short interval means ranged from 11 to 35ms and long interval means ranged from 48 to 184ms. Short and long intervals differed significantly in 33 of 39 cases. However, in one of 22 flights and five of 17 pendulum swings, short and long intervals were not significantly different ($P > 0.05$). A significant relationship exists between the experimental situations for interpulse intervals.

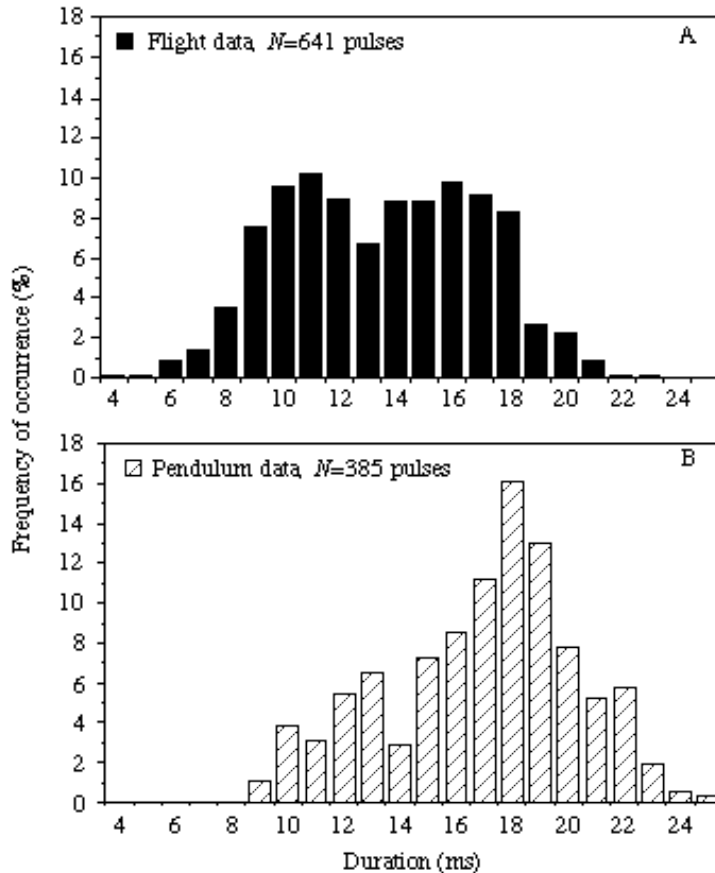


Fig. 6. Histogram of pulse durations from all four bats in flight (A) and on the pendulum (B).

In a greater proportion of flights, long and short interval groups were significantly different compared with interval groups on the pendulum ($\chi^2=4.54$, d.f.=1, $P<0.05$). Short intervals in flight differed from short intervals on the pendulum in all four bats ($P<0.01$). Flight and pendulum behavior did not separate as well on the basis of long intervals. In two animals long intervals between flight and pendulum differed significantly ($P<0.01$), and in two others there was no significant difference.

Discussion

This study demonstrates the utility of vocalization radiotelemetry in the investigation of echolocation behavior in flying bats. We monitored echolocation activity of bats in flight, without the constraints of simulated flight. Telemetry seems particularly useful for CF-FM bats as a method of recording pulse timing and emission frequencies free from Doppler shifts. The 0.85g transmitter, which represented approximately 7% of the bat's body weight should result in a proportional decrease in maneuverability (Aldridge and Brigham, 1988). However, experiments by Evans and Thomas (1992) have demonstrated

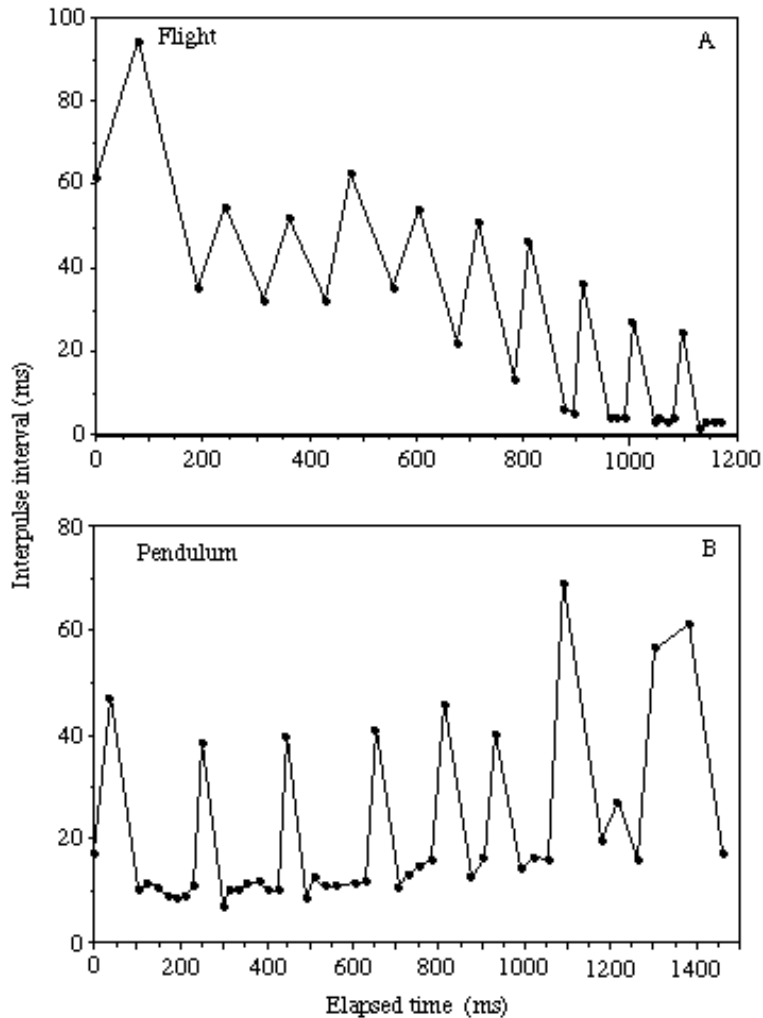


Fig. 7. Interpulse intervals during a landing sequence (A) and on the pendulum (B) for bat YR. Note that repetition rate steadily increases and interval durations decrease during approach to the landing. In the pendulum swing, high repetition rates often occur early and durations of intervals do not decrease steadily. These sequences are considered to be representative of most flights and pendulum swings.

that a weight placed away from a bird's center of gravity can have a disproportionate effect. Nevertheless, we detected no perceptible changes over periods of less than 1h.

A primary goal of this study was the comparison of Doppler-shift compensation behavior between free flight and simulated flight on a pendulum. Both experimental systems have advantages and disadvantages. During simulated flight, vocalizations and multiple biopotentials may be simultaneously monitored, along with changes in the position and velocity of the pendulum (Henson *et al.* 1982). Hard-wired recordings provide superior quality and better signal-to-noise ratio than telemetered signals. Despite

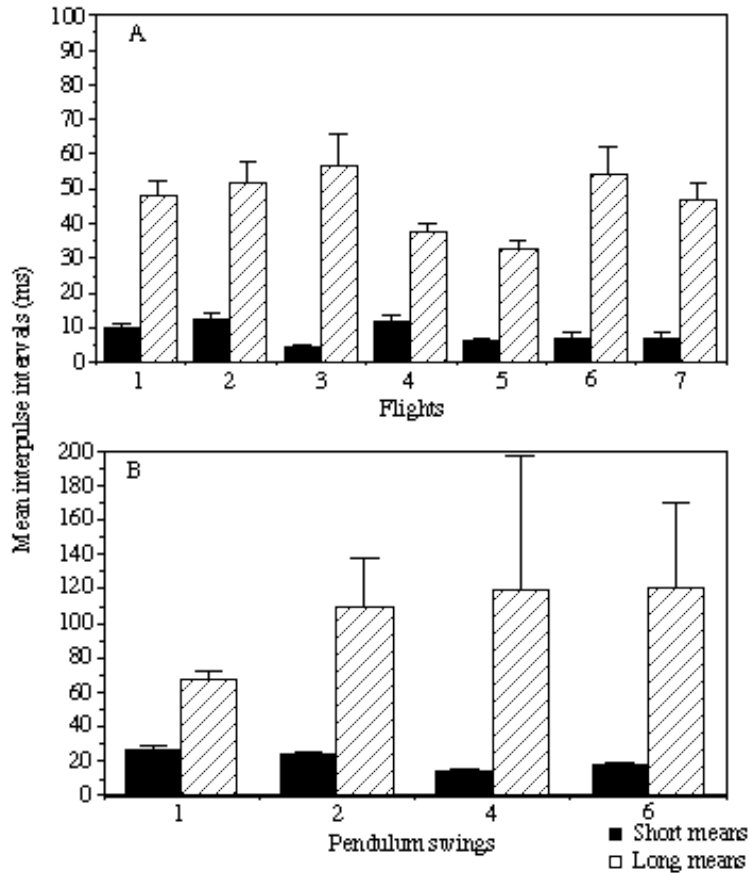


Fig. 8. Histogram of short and long interpulse intervals of bat WL in flight (A) and on the pendulum (B). Individual flights and pendulum swings are depicted separately with error bars representing standard error of the mean. Note the difference in scales. These data are considered to be representative of the bats we tested.

disadvantages (reception nulls, variable signal-to-noise levels and reduced dynamic range), telemetry offers the only means of recording vocalizations in true flight where the recording microphone is in a constant position relative to the head. Thus, the precise time of pulse emission can be recorded.

Differences were found between free flight and pendulum swings in three parameters of echolocation: pulse duration, frequency control and interpulse interval. Longer constant-frequency pulse durations were used on the pendulum than in free flight. Durations in flight ranged from 4 to 23ms (mean 13.2ms), while durations on the pendulum ranged from 9 to 25ms (mean 16.8ms). Part of this difference could be attributed to the lack of clear terminal phase behavior in pendulum swings. In landing sequences in a familiar setting, however, the bats seldom used extended terminal phases and, in many flights, the bats did not land but turned at the target. Terminal phase emissions occurred in nine of the 22 flights constituting our data set. When terminal

Table 3. Summary of interpulse interval data

		Bat	Mean interval (ms)	Standard error	N †
Short intervals	Pendulum	NB	12.6	0.64	61 *
	Flight		9.1	0.09	23
Long intervals	Pendulum		123.2	33.18	36
	Flight		64.0	10.91	27
Short intervals	Pendulum	WL	18.5	1.45	19 *
	Flight		7.7	0.58	57
Long intervals	Pendulum		102.2	14.11	68 *
	Flight		44.9	2.09	119
Short intervals	Pendulum	WR	14.6	0.53	60 *
	Flight		10.7	0.57	76
Long intervals	Pendulum		55.2	3.55	51
	Flight		55.8	5.16	86
Short intervals	Pendulum	YR	13.2	1.15	42 *
	Flight		7.8	0.49	116
Long intervals	Pendulum		113.5	28.66	29 *
	Flight		49.8	2.13	115

* Flights and pendulum swings significantly different, $P \leq 0.01$.

† Number of intervals is equal to one less than the number of pulses per flight multiplied by the number of flights (see Table 1).

phases did occur, constant-frequency pulse durations ranged from 6.2 to 12.8ms (mean 9.99 ± 1.93 ms, $N=81$). With terminal phase durations removed from the data set, durations from flight and pendulum swings were still significantly different. Therefore, the inclusion of short, terminal phase pulses in the data set was not responsible for the differences in duration between the two experimental situations.

Duration of the constant frequency pulse has been considered to be of importance in the accuracy of Doppler-shift compensation. Schuller (1977) found a minimum duration of approximately 10ms necessary for *Rhinolophus ferrumequinum* to Doppler-shift compensate accurately. In our experiments, *P. parnellii* used a longer mean duration on the pendulum than in flight, but the longer duration did not result in improved compensation.

We found a significantly greater precision in frequency control in freely flying bats versus bats on a pendulum (Fig. 5, Table 1). As previously noted, 59% of all echoes fell into a range of 150Hz around reference frequency in flight and 93% occurred in a range of 500Hz. On the pendulum, 23% fell in the 150Hz range and 72% in the 500Hz range. For each bat individually, the standard error of echoes in flight was less than on the pendulum and mean echo frequency in flying bats was significantly different from recordings made on the pendulum (Table 2). Studies which have addressed the accuracy of frequency control in Doppler-shift compensating bats, particularly in *P. parnellii*, may also have been compromised by experimentation with restrained animals. To assess the accuracy of Doppler-shift compensation, most other studies have reported the degree of

pulse frequency shift corresponding to a known velocity or simulated echo frequency (Schuller *et al.* 1975; Gaioni *et al.* 1990). Accuracy is expressed as a percentage of complete compensation. This convention makes comparison of their results with ours difficult. In our experiments, both echo frequency and velocity were calculated from frequency differences between the telemetered signal and the stationary microphone. Comparison of velocity to any frequency value constitutes circular logic. Without an independent measure of velocity, we were unable to describe accuracy in this way.

Interpulse interval patterns can yield information on repetition rate, pulse grouping and breath control. Trends in our data can be attributed to differences in the experimental systems. Long intervals represent the time between breaths or cessation of emissions. In a single flight or pendulum swing, great variations in the length of long intervals indicates a change in breathing rhythm, a lack of challenge in the echolocative task, or both. Although our flight conditions did not present the bats with a complex challenge, any flight represents a more natural situation than simulated flight. Two of the four bats showed significant differences between long interpulse intervals in flight compared to pendulum swings (Table 3). In both cases, long interpulse intervals on the pendulum were approximately twice as long as in flight and had larger standard errors. In the case of bat NB, long intervals were different at a lower level of confidence ($P < 0.1$), but showed similar relationships. Short interpulse intervals were significantly shorter in flight than on the pendulum in all bats ($P < 0.01$) (Table 3). Means of short intervals in flight ranged from 43 to 73% of the means of short intervals on the pendulum, but the standard errors were more similar than in long intervals. As in pulse durations, in flights with terminal phase emission sequences, the higher repetition rate could skew the intervals to a lower mean. However, in comparisons of flights without terminal phases with pendulum swings, a statistically significant difference remains ($P < 0.05$). Therefore, as in duration, differences in interpulse intervals between true flight and simulated flight exist in search and approach phases and are not a product of the lack of terminal phase emissions on the pendulum.

Interpulse interval patterns have been studied previously and dual populations of long and short interpulse intervals have been reported in many bats (Joermann and Schmidt, 1981; Jen and Kamada, 1982; Hartley *et al.* 1989; Thomas *et al.* 1990). In flying *Phyllostomus hastatus*, alternating short and long intervals between individual pulses and groups of pulses have been correlated with the respiratory cycle (Suthers *et al.* 1972). The differences seen in the pattern of interpulse intervals between flight and pendulum swing in *P. parnellii* may also be attributable to a pattern of respiration in flight that is not imposed on restrained animals.

The linkage between wingbeat, respiration and echolocation pulse emission was demonstrated by Suthers *et al.* (1972), and Roberts (1972) correlated respiration and ultrasound production in bats. Grinnell and Griffin (1958) suggested that pulse grouping could correlate with the respiratory cycle and Griffin *et al.* (1960) discussed the correspondence between wingbeat and pulse emission. Experiments on *Myotis lucifugus*, *Pteronotus parnellii rubiginosa*, *Carollia perspicillata* and *Rhinolophus ferrumequinum* by Schnitzler (1971, 1973) revealed that all of these bats emit a single sound or group of sounds per wingbeat, and he postulated a probable correlation between respiration and

wingbeat. Joermann and Schmidt (1981) correlated wingbeat and vocalization in *Desmodus rotundus*, finding that the first pulse was emitted at the beginning of the upstroke and the pause in vocalization was seen during the downstroke.

Coordination between limb movement and respiration is not unique to bats. Locomotion/respiration coupling has been demonstrated in many mammals and birds and has been credited with the sustained energetic activities in which mammals and birds engage (Berger *et al.* 1970; Bramble and Carrier, 1983; Alexander, 1989). The production of vocalizations by respiratory air movements links echolocation in bats to locomotion/respiration coupling. During echolocation, bats produce the energy used to image their environment. The production of intense vocalizations at a high rate of repetition, which for most mammals is an energetically costly activity, has been integrated with locomotion and respiration with no net increase in energetic cost (Speakman and Racey, 1991). This is one of the most fundamental advances by which bats have flourished in the niche of aerial, nocturnal predators and foragers. Experiments that rely on flight simulations have the effect of decoupling respiration and hence vocalization from locomotion. These effects have not been addressed in experiments where restrained bats were induced to vocalize.

Since respiration and vocalization have been correlated with the wingbeat cycle, it is clear that experiments using restrained animals in echolocative studies have removed the essential mechanism that controls timing of pulse emission. Accurate characterization of timing becomes especially important in attempts to understand the changes in strategy as a CF-FM bat approaches a target. Each wingbeat presents a window of opportunity for vocalization. Pulse repetition rate increases while the duration of the CF decreases. The FM duration also decreases, but by a proportionately smaller amount (Henson *et al.* 1987). In the terminal phase of echolocation, *P. parnellii* reduce calls to a series of brief pulses, consisting of an upward-sweeping initial FM followed by a downward-sweeping terminal FM; the CF component is practically eliminated. These changing variables are basically changes in timing. Experimental protocols that disrupt the essential mechanism of timing cannot replicate the normal behavior.

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