

Variations in respiratory muscle activity during echolocation when stationary in three species of bat (Microchiroptera: Vespertilionidae)

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Summary

Echolocating bats use respiratory muscles to power the production of biosonar vocalisations. The physical characteristics of these calls vary among species of bat, and variations also exist in the timing and patterns of respiratory muscle recruitment during echolocation. We recorded electromyograms from the respiratory muscles of three species of bat (Family Vespertilionidae) while the animals vocalised from stationary positions. Activity was recorded consistently from the lateral abdominal muscles (internal abdominal oblique and transversus abdominis) from all calling bats, but we found much variation within

and among species. Bats in the family Vespertilionidae devoted longer periods of expiratory muscle activity to each call than did the mormoopid bat *Pteronotus parnellii*. These differences correlate negatively with the duration of calls. We suggest that morphological adaptations in some bats may facilitate the economic production of echolocation calls at rest.

Key words: bat, *Eptesicus serotinus*, *Pipistrellus pygmaeus*, *Myotis myotis*, echolocation, respiration, Vespertilionidae, vocalisation, electromyography.

Introduction

The structure of biosonar signals used by bats varies widely in duration, intensity, frequency structure and duty cycle. Calls range from less than 1 ms in duration to more than 50 ms (Novick, 1971; Jones and Rayner, 1989). Intensity varies over two orders of magnitude (Waters and Jones, 1995). Frequency structure ranges from the calls of the spotted bat *Euderma maculatum* that are clearly audible to humans (Woodsworth et al., 1981), to calls in excess of 200 kHz in *Clootis percivali* (Fenton and Bell, 1981). Duty cycle varies widely with differences in duration and repetition rate.

Differences in the physical characteristics of biosonar signals are reflected in the varying biosonar strategies and the diversity of lifestyles of echolocating bats. Differences are also reflected in the morphology and function of the respiratory system. The morphology of the respiratory muscles and related structures in bats was described by Lancaster and Henson (1995), and adaptations were found that could enhance the performance of the respiratory system for vocalisation. The abdominal wall surface is dominated by a dense, bilaminar aponeurosis from which the fascicles of the internal abdominal oblique and transversus abdominis radiate to attachments on the ribs, thoracolumbar aponeurosis and pelvis. In its most derived condition, this structure resembles the diaphragm and is considered to be functionally analogous. Differences were noted between species that employ differing biosonar strategies, suggesting that the physiological demands of producing some types of call might be greater than for others.

Speakman et al. (1989) measured the oxygen consumption

of stationary, echolocating pipistrelle bats (*Pipistrellus pipistrellus*=*Pipistrellus pygmaeus*) and found a high cost for the production of individual calls in a stationary bat. The cost, extrapolated to the rate of 10 calls s⁻¹ commonly seen in flight, could amount to an average of 9.5 times the basal metabolic rate. These bats, however, were reported to be reluctant to vocalise at rest, similar to the behaviour reported by Roberts (1972) in *Eptesicus serotinus* and *Plecotus auritus* and perhaps reflecting the costs of frequent vocalisation in a stationary situation. This contrasts sharply to the behaviour of *Pteronotus parnellii* (Lancaster et al., 1992, 1995) and species of *Rhinolophus* and *Hipposideros* (Roberts, 1972; Link et al., 1986; Jones and Rayner, 1989) that freely produce biosonar vocalisations at rest. How can these bats produce large numbers of echolocation calls at rest if the costs are as great as some studies suggest?

The activity of respiratory and flight muscles in relation to vocalisation in stationary and flying bats was studied by Lancaster et al. (1995), who found bursts of activity in the flank muscles (lateral abdominal wall) corresponding to each individual vocalisation in *Pteronotus parnellii*, both at rest and during flight. To address this paradox between high energetic costs of echolocation at rest in one species and vigorous muscular activity associated with it in another, we undertook a study of the muscular activity associated with vocalisation in several species of bat that use different types of biosonar calls and display a range of vocal behaviours. Here, we describe the activity patterns of muscles recruited during vocalisation in

three species of bat at rest. We will consider differences between these patterns and how they relate to the strategy of echolocation and the physical characteristics of the calls.

Materials and methods

Three species of bat were used for these experiments: five female *Eptesicus serotinus* (Schreber) from southern England, four *Pipistrellus pygmaeus* (Leach) from northern Scotland and four *Myotis myotis* (Borkhausen) from Switzerland. These are relatively small numbers of animals; our sample sizes were constrained by the protected status of bats in Europe and limitations of the licences permitting us to take animals from the wild. All bats were captured, transported and held in captivity under permit from appropriate regulatory authorities. Animals were maintained in captivity on a diet of meal worms (*Tenebrio* larvae), crickets (for *M. myotis*) and vitamin supplements. Care was under the supervision of the Home Office and in compliance with the Animal Welfare Act. Animals were housed in an open-air enclosure with adequate space for flight and provided with both heated and ambient-temperature roost boxes. Formalin-fixed and skeletonized specimens of all species were used for morphological studies.

Electromyography

Electromyographic and surgical protocols were adapted from the procedures of Lancaster et al. (1995). In each species, electromyograms were recorded from the lateral abdominal wall or flank muscles (*m. obliquus internus abdominis* and *transversus abdominis*) (Nomina Anatomica Veterinaria, 1983), pectoralis (*m. pectoralis*) and serratus ventralis (*m. serratus ventralis*). In *E. serotinus* and *M. myotis*, we recorded the activity of the costal diaphragm (*m. diaphragma pars costalis*), the costal portion of the external abdominal oblique (*m. obliquus externus abdominis*) and the rectus abdominis (*m. rectus abdominis*) (*E. serotinus* only). Table 1 lists the number of implants in each muscle for each species. Because of the unusual morphology of the abdominal musculature and aponeurosis (described above), the internal abdominal oblique and transversus abdominis are considered to have a common function and, henceforth, we refer to the two muscles collectively as the lateral abdominal wall. Recordings from the lateral abdominal wall and costal portion of the external oblique were not selective of specific muscle layers. Because

of its thinness and distribution, the abdominal portion of the external abdominal oblique is not considered to be a major contributor to the signals recorded from the flank muscles. The combined activities of the internal oblique and transversus abdominis are considered to constitute the majority of the signal. Recordings from the costal musculature were made from the region lateral to the sternum where the costal portion of the external abdominal oblique predominates.

Electrodes

Recordings from the abdominal and thoracic muscles and diaphragm were made with patch electrodes constructed from Silastic sheeting and insulated, silver bipolar electrode leads (76 µm diameter; interelectrode distance approximately 2 mm). Electrodes for the rectus abdominis, pectoralis and serratus ventralis were of the offset hook design with 0.5 mm bare areas separated by 0.5 mm (Loeb and Gans, 1986).

Surgery

Surgical procedures were in accordance with established animal care guidelines. Under general anaesthesia (Halothane, 2.0% at 1 l min⁻¹), patch electrodes were attached to the surface of the abdominal wall with 7-0 synthetic sutures. In approaching the diaphragm, incisions into the abdominal wall were made at the mid-axillary line, 2–3 mm caudal to the costal margin to avoid the pleural cavity. The patch electrode was inserted between the liver and the diaphragm, with the receptive surface oriented in contact with the diaphragm. To approach the costal portion of the external oblique, the fascial sheath attaching the caudal edge of the pectoralis to the surface of the ventral abdominal wall was bluntly dissected lateral to the sternal attachments. The pectoralis was then elevated, and the patch electrode was inserted beneath with the receptive surface in contact with the underlying external oblique. The Silastic patch served as insulation between the electrode and the overlying pectoralis. Hook electrodes were inserted into the pectoralis and serratus ventralis with a 27 gauge hypodermic needle, and the leads were secured to fascia with a suture.

Connectors were made either from single-row, right-angled, square-post headers (2.5 mm centre space) or from the edges of thin, printed circuit card bus connectors. Electrode leads were soldered to the terminals, and the connector was attached to a 10 mm×20 mm Silastic patch using silicone adhesive. Bus connectors had the advantage of quickly releasing if the animal pulled at the recording leads. After implantation, the skin was closed over the connector patch. Sutures (6-0 silk) were used to secure the entire assembly to the skin. In *E. serotinus* and *M. myotis*, two muscles were implanted in each animal; because of its small size, only one muscle was implanted in each *Pipistrellus pygmaeus*. Recordings began on the day following surgery to allow the animals to recover fully from anaesthesia. The implants remained in the animals for 3–7 days. After completion of recordings, the animal was anaesthetized, and the position of electrodes was verified by dissection.

Table 1. Number of implants into individual bats by species and muscle in this study

Muscles	<i>Eptesicus serotinus</i>	<i>Myotis myotis</i>	<i>Pipistrellus pygmaeus</i>
External oblique	3	2	0
Pectoralis	2	1	1
Diaphragm	2	1	0
Abdominal wall	3	2	2
Rectus abdominis	1	0	0
Serratus ventralis	1	1	1

Recording

All electromyographic recordings were made in conjunction with echolocative vocalisations. Subjects were placed in a small, half-cylindrical cage (35 cm long by 25 cm in diameter) with a plastic floor. A shielded cable (NMUF 4/30, Cooner Wire Co., Chatsworth, CA, USA) connected the implant to a custom-made, two-channel instrumentation amplifier (gain 200, bandpass filter 1–3 kHz, based on AD625 instrumentation operational amplifier; Analog Devices, Norwood, MA, USA). A model 2630 (6.25 mm) Larson Davis condenser microphone with model 2200C Larson Davis amplifier received the vocalisations (through a 15 kHz highpass filter). All signals were recorded on a four-channel Racal instrumentation tape recorder at 38 cm s^{-1} . The subject-to-microphone distance was sufficiently small (typically 25 cm or less, maximum possible 40 cm) that time delays were negligible. Recordings were made while the animal hung in the cage, free to move about; occasionally, *M. myotis* rested horizontally on the cage floor. Voiced comments describing the bat's activity were made on the tape. Sessions typically lasted less than 30 min.

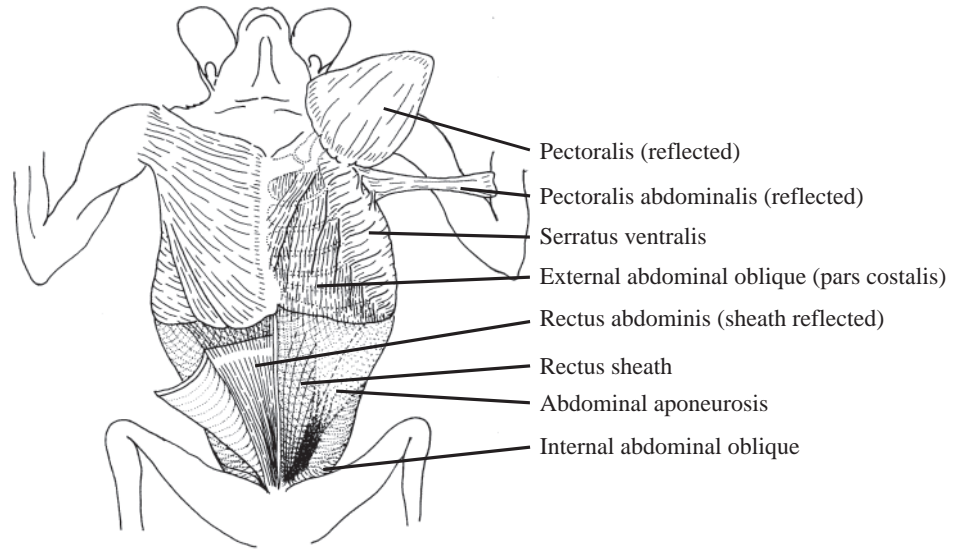


Fig. 1. Ventral view of the musculature of the body wall of *Pipistrellus pygmaeus*.

Analysis

Selected recordings were replayed at half speed into a National Instruments AT MIO 16E2, 500 kHz A/D board and digitally recorded with a custom-designed data-acquisition routine run on Labview 3.1.1. Continuous sequences of 2 s duration were digitized at 160 kHz and displayed on a digital oscilloscope. Aspects of pulse and myopotential timing were tabulated by measuring sequential onsets and terminations of

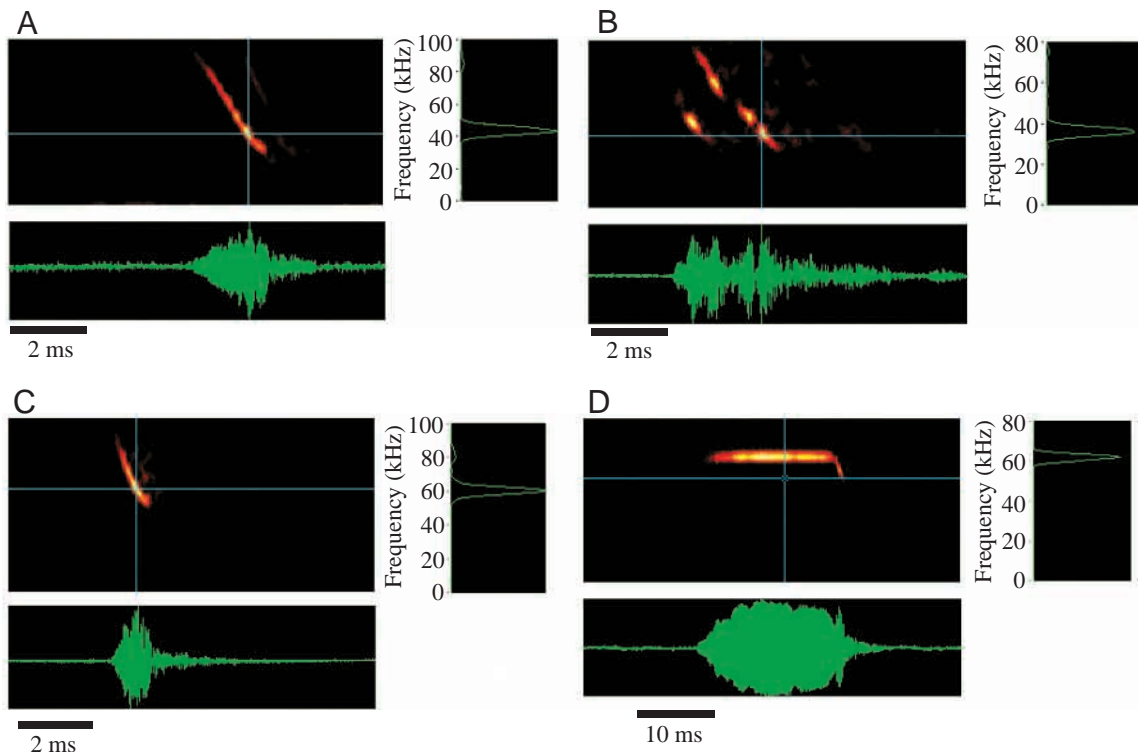


Fig. 2. Sonograms (top left), oscillograms (bottom) and Fast Fourier transforms (top right) of biosonar calls recorded at rest. (A) *Myotis myotis*; (B) *Eptesicus serotinus*; (C) *Pipistrellus pygmaeus*; (D) *Pteronotus parnellii*.

pulses and myopotentials using the custom-made Muscle and Vocal Responses Detector software (Vertron Ltd, Dunfermline, Scotland) run on Labview. The routine searched 200 ms segments of a file for signals on the vocal channel exceeding an adjustable threshold. Upon detection, the active cursor searched for a signal on each muscle channel within a predefined time window. Active cursors detected both the onset and offset of signals on the basis of the preset threshold; subthreshold gaps of 1.25 ms within a myopotential were allowed before resetting for detection. Threshold was left at the default setting but could be adjusted manually in the case of a sequence with poor signal-to-noise ratio. The values of all six active cursors (onset and offset of the vocal and two muscle channels) were saved by the operator, and detection was then reset. Data were later analysed in a spreadsheet program (Microsoft Excel).

We calculated the following parameters from the raw data: pulse duration, myopotential duration for both muscles, interpulse interval and intermyopotential interval. Specific parameters were quantified for individual muscles. Periodic repetition rates for the diaphragm and expiratory muscles (equivalent to respiratory rate) and respiratory period were calculated. Means of variables were calculated for each measured sequence and used to calculate overall means \pm S.E.M. for all sequences for each bat.

We compared the durations of different events across individuals using one-way analysis of variance (ANOVA). Individual differences were generally not significant, so we pooled all the data within a given species for further analysis. The durations of given myopotentials and vocalisations were all approximately normally distributed and, if not, were transformed prior to analysis. We compared the durations of different events within breaths using paired *t*-tests. We examined the associations between different events using χ^2 analysis.

We expressed the relative muscular effort devoted to the generation of vocalisations across the four species by plotting the duty cycle of abdominal wall contraction with respect to the duty cycle of vocalisation as a bar graph (see Fig. 7).

Values presented in the text and tables are means \pm S.E.M.

Results

Fig. 1 is a ventral view of a dissection of *Pipistrellus pygmaeus* depicting the morphology of the thoracic and abdominal walls. Most gross features are consistent with previous descriptions of Microchiroptera (Vaughan, 1970b; Lancaster and Henson, 1995) with the exception of the external abdominal oblique. This muscle is similar in its orientation and attachments to that in other mammals (Winckler, 1950) except that it is divided into separate costal and abdominal portions. From origins that interdigitate with the serratus ventralis, six costal bands insert into the fascia and surface of the abdominal aponeurosis at the costal margin. Abdominal fascicles arise separately from the costal margin and course caudally and ventrally to attachments on the pelvis.

Separation of this muscle into two parts occurs in all three species under study and also in specimens of *Pteronotus parnellii* and *Rhinolophus ferrumequinum* that we examined. All the other muscles from which we recorded are comparable with those of *Pteronotus parnellii* as described by Lancaster et al. (1995).

Electromyography and vocalisation

Vocalisations

The muscles from which electromyograms were recorded and the number in each species are listed in Table 1. Bats displayed variable vocal behaviour when confined in the small recording cage. Of the three species, *Pipistrellus pygmaeus* produced the fewest biosonar vocalisations, *E. serotinus* was intermediate and *M. myotis* produced the greatest number of calls. We performed a spectral analysis of a sub-sample of calls that had a good signal-to-noise ratio. These calls had a structure and frequency content (Fig. 2) typical of these species (Kalko and Schnitzler, 1993; Jensen and Miller, 1999; Ahlén and Baagøe, 1999), although calls were typically of shorter duration than the biosonar calls used in flight. For *Pipistrellus pygmaeus*, we analysed 55 breath cycles, including 31 cycles in which the animal produced vocalisations (Table 2). The duration of the first vocalisation averaged 2.16 ± 0.12 ms ($N=31$). When double calls were produced (12 of 31 cycles), the second calls were significantly longer ($t=4.49$, $P=0.0001$, d.f.=40) and more variable in duration.

In the set of 182 respiratory cycles analysed for *E. serotinus*, the bats only produced single calls, although we occasionally recorded paired calls like those produced by pipistrelles (Fig. 3B,D). Calls emitted by *E. serotinus* were slightly and significantly longer than the first calls produced by *Pipistrellus pygmaeus*, averaging 2.71 ± 0.135 ms ($N=145$) (difference between *Pipistrellus pygmaeus* and *E. serotinus*; $t=2.34$, $P=0.01$, d.f.=174). The calls produced by *M. myotis* were also short in duration (mean 2.88 ± 0.088 ms, $N=451$). This was not significantly different from the calls produced by *E. serotinus* ($t=1.07$, $P=0.29$) but was significantly longer than calls produced by *Pipistrellus pygmaeus* ($t=2.62$, $P=0.0091$, d.f.=550). *M. myotis* emitted single and double pulses, but unlike the other species often produced calls in groups of four or more (see Fig. 5D). In one instance in the quantified data set, a bat produced a group of 28 calls within a single breath cycle at a repetition rate of $150 \text{ pulses s}^{-1}$.

Muscle activity

Rectus abdominis

Recordings from the rectus abdominis of *E. serotinus* showed no activity correlated with biosonar vocalisation (Fig. 3D), and no further recordings were made.

Lateral abdominal wall

The mean duration and relative time of onset of myopotentials in the abdominal wall and associated calls are given in Table 2. Fig. 3A–C depicts examples of the activity of the lateral abdominal wall in relation to vocalisation in

Table 2. Myopotential and vocalisation durations and the onset times of myopotentials and vocalisations in three species of vespertilionid bat

	Onset time (ms)	Duration (ms)	<i>P</i>	<i>N</i>	<i>N_b</i>		Onset time (ms)	Duration (ms)	<i>P</i>	<i>N</i>	<i>N_b</i>
<i>Myotis myotis</i>						<i>Pipistrellus pygmaeus</i>					
Vocalisation						Vocalisation					
1	11.58±0.854	2.229±0.097	0.91	85	93	1	10.95±1.78	2.16±0.12	0.56	31	55
2	22.94±1.16	2.729±0.089	0.97	90	93	2	40.34±6.38	5.76±1.35	0.22	12	55
3	30.26±1.18	3.075±0.094	0.91	85	93	Abdominal wall					
4	40.74±2.20	3.242±0.294	0.76	71	93	Contraction 1	0	12.73±1.10	1.0	55	55
5	53.13±2.46	2.924±0.121	0.54	50	93	Contraction 2	22.08±1.61	12.73±0.70	0.8	44	55
6	62.02±3.29	2.992±0.149	0.42	39	93	Contraction 3	53.0±19.9	11.35±3.3	0.04	2	55
7	68.94±4.30	2.167±0.241	0.35	33	93	Pectoralis					
Abdominal wall						No coordinated contraction reported					
Contraction 1	0	6.253±0.394	1.0	75	75	Serratus ventralis					
Contraction 2	13.73±0.842	4.188±0.225	0.92	69	75	No coordinated contraction reported					
Contraction 3	21.15±0.606	3.733±0.171	0.8	60	75	<i>Eptesicus serotinus</i>					
Contraction 4	31.00±1.15	3.154±0.168	0.52	40	75	Vocalisation					
Contraction 5	36.73±0.605	3.568±0.261	0.49	37	75	1	19.81±0.847	2.71±0.135	0.90	145	161
Contraction 6	44.57±0.782	3.758±0.265	0.44	33	75	Abdominal wall					
Contraction 7	52.21±1.19	3.714±0.256	0.37	28	75	Contraction 1	0	10.33±0.371	1.0	161	161
External oblique						Contraction 2	26.79±1.43	12.12±0.842	0.53	85	161
Contraction 1	-2.48	7.22±0.703	1.0	18	18	External oblique					
Contraction 2	11.02±0.54	5.611±0.657	1.0	18	18	Contraction 1	-1.74±0.447	11.48±0.477	1.0	107	107
Contraction 3	21.24±1.48	4.444±0.544	1.0	18	18	Contraction 2	11.02±0.54	5.61±0.657	0.65	70	107
Contraction 4	36.31±2.18	3.437±0.491	0.89	16	18	Diaphragm					
Contraction 5	50.50±11.30	4.25±1.10	0.44	8	18	Contraction 1	15.19±0.987	4.93±0.987	0.95	20	21
Contraction 6	75.0±33.7	3.33±0.33	0.16	3	18	Contraction 2	40.91±5.48	35.96±3.25	1.0	21	21
Contraction 7	91.7±31.5	4.67±1.2	0.16	3	18	Pectoralis					
Diaphragm						No coordinated contraction reported					
Contraction 1	80.85	37.38±2.23	1.0	66	66	Serratus ventralis					
Contraction 2	23.37±6.92	3.55±0.38	0.30	20	66	No coordinated contraction reported					
Contraction 3	58.47±11.5	6.50±2.5	0.03	2	66	Rectus abdominus					
Pectoralis						No coordinated contraction reported					
No coordinated contraction reported											
Serratus ventralis											
No coordinated contraction reported											

In all three species, the data presented are mean ± s.e.m.

P represents the probability of that event occurring in a given respiratory cycle.

All onset times are defined relative to the onset of the first myopotential of the abdominal wall in each cycle.

The *N* value shows the total number of events on which the calculated means and standard errors are based, and *N_b* refers to sample size of respiratory cycles used for analysis of any particular trait.

For *Myotis myotis*, the data are based on a total of 144 respiratory cycles across four individuals. The mean duration of the respiratory cycles was 136.98±4.56 ms (*N*=131).

Data for *Pipistrellus pygmaeus* are based on 55 breaths across two individuals. In this species, mean duration of respiratory cycles averaged 74.66±2.49 ms (*N*=47). For *Eptesicus serotinus*, the data are based on 182 respiratory cycles. In this species, the mean duration of the respiratory cycle was 96.26±4.14 ms (*N*=148).

E. serotinus, and these data are summarised in Fig. 6B. Abdominal wall myopotentials were much longer than vocalisations. The distribution of durations of myopotentials of the abdominal wall was positively skewed, with most lasting between 7 and 14 ms (mean 10.95±0.382 ms) but some lasting up to 30 ms. In 85 of the 161 abdominal wall cavity cycles analysed, the bats had two bursts of activity in the abdominal wall separated by an interval of approximately 16 ms; in the remainder, only a single burst was recorded. The duration of

the first contraction did not differ significantly from that of the second contraction ($t=-0.27$, $P=0.79$, d.f.=244) when the distribution of durations of myopotentials had been normalised by logarithmic conversion before testing.

When vocalisations occurred (*N*=145), they generally started after the first contraction had ended, with only 6.6% starting before termination of the abdominal wall contraction. There was no significant relationship between the duration of the first abdominal wall contraction and the duration of

the vocalisation that followed ($r^2=0.014$, $P>0.05$) and there was also no relationship between the duration of the first abdominal wall contraction and the presence or absence of a following vocalisation (one-way ANOVA; $F_{1,149}=0.16$, $P=0.69$). When the bats vocalised, a second burst of activity occurred in the abdominal wall on 59% of occasions; when they did not vocalise, a second burst of activity was infrequent (7% of cases). Because they produced two bursts more frequently when they vocalised, the total duration of abdominal wall activity was significantly greater in the presence of vocalisations (Table 3) (one-way ANOVA; $F_{1,159}=4.82$, $P=0.03$).

The pattern of activity in the abdominal wall of *Pipistrellus pygmaeus* resembled that of *E. serotinus* (Figs 4A,B, 6C) (Table 2). In 44 of 55 breath cycles, these bats produced a second burst of abdominal wall activity, and on two occasions produced a third. Similar to *E. serotinus*, contractions of the abdominal wall were much longer than the duration of vocalisations and the frequency distribution was also positively skewed, ranging from 4 to 32 ms in duration. There was no significant difference in the durations of the myopotentials between the first and second bursts of activity (two-sample t -test; $t=0$, $P>0.05$, d.f.=97) after the distributions had been normalised by logarithmic transformation.

There were, however, important differences between *Pipistrellus pygmaeus* and *E. serotinus*. In *Pipistrellus pygmaeus*, 17 of 31 calls commenced before the first abdominal wall contraction had finished. There was no association between the occurrence of a second contraction of the abdominal wall and the presence or absence of a vocalisation ($\chi^2=0.296$, d.f.=1, $P>0.05$). Succeeding myopotentials accompanied 24 of 31 respiratory cycles in which a vocalisation occurred. However, in the absence of a call, double myopotentials occurred in 20 of the 24 cases. The durations of these second contractions bore no relationship with the presence or absence of a vocalisation ($F_{19,23}=1.66$, $P=0.204$), but the duration of the vocalisation was strongly related to the presence or absence of a succeeding burst of activity. Calls that were succeeded by a myopotential were significantly longer (2.35 ± 0.116 ms, $N=24$) than those that

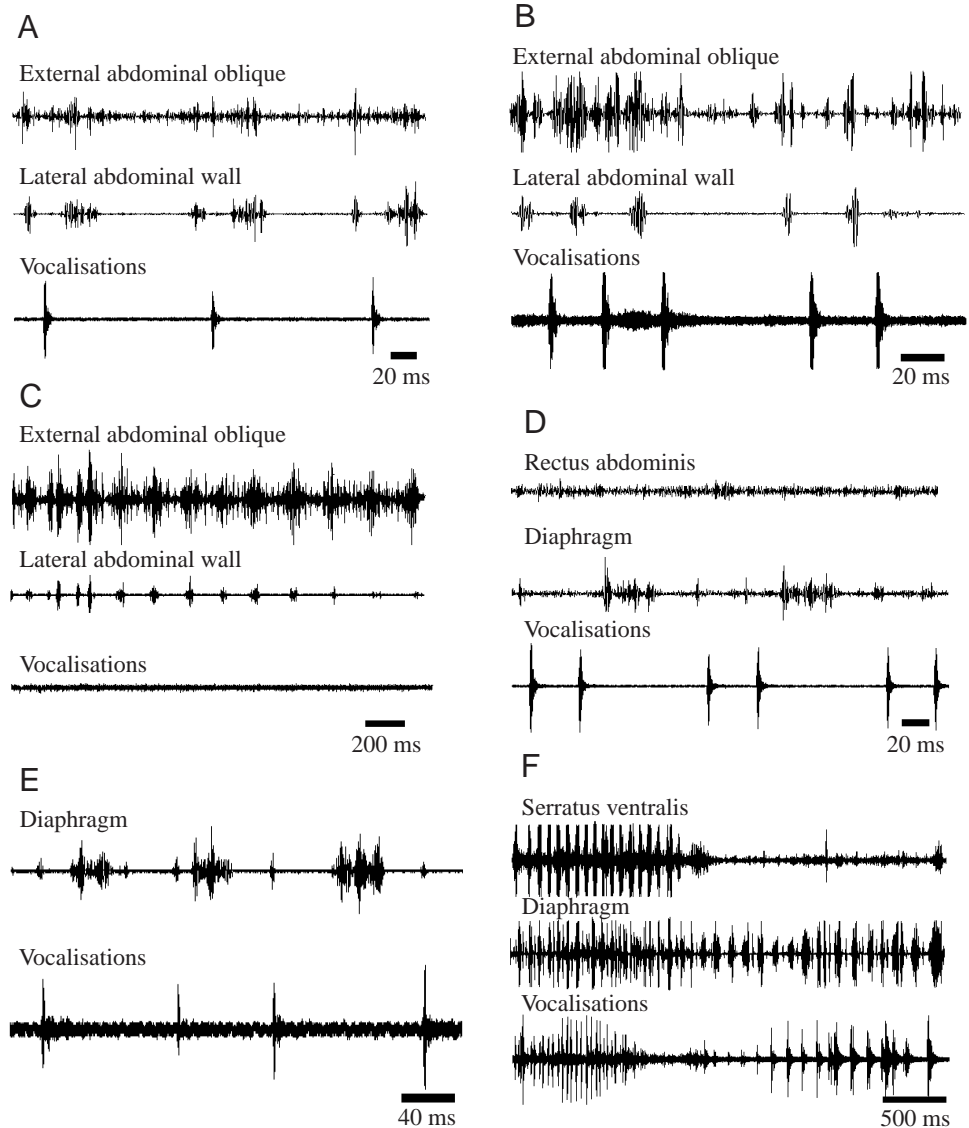


Fig. 3. Electromyograms recorded with respect to vocalisation from *Eptesicus serotinus* at rest.

were not (1.33 ± 0.71 ms, $N=7$; one-way ANOVA; $F_{1,29}=19.15$, $P<0.0001$). The presence of a second vocalisation ($N=12$ breath cycles) occurred only when there was a second abdominal wall contraction, although there were often second abdominal wall contractions without second vocalisations ($N=32$). The presence of the second vocalisation was not related to the duration of the second abdominal wall contraction (one-way ANOVA; $F=2.01$, $P=0.164$).

Activity in the lateral abdominal wall in *M. myotis* resembled that in the other species (Figs 5A, 6A) (Table 2); however, the distribution of abdominal wall myopotential durations was normal rather than positively skewed. The average duration of activity in the abdominal wall was 4.48 ± 0.159 ms ($N=256$), significantly shorter than in the other two species (one-way ANOVA of abdominal wall contraction durations of the three species; $F=7.45$, d.f.=2,694, $P=0.0028$; comparison of *M. myotis* with *E. serotinus*, $t=4.81$, $P=0.003$; comparison of *M. myotis* with *Pipistrellus pygmaeus*, $t=5.67$,

$P=0.0001$). Consistent with vocal behaviour, abdominal wall activity often occurred as a series of high-repetition-rate bursts. There was a close correspondence between the number of abdominal wall bursts in a breath cycle and the number of vocalisations. Each vocalisation was associated with a single burst in the abdominal wall, but the timing of the association was not consistent (Fig. 5A). In general, the vocalisations tended to lag behind the corresponding burst of activity in the abdominal wall, and this lag became progressively greater as the number of vocalisations increased (Table 2) (Fig. 6A). There was a significant decrease in the duration of abdominal wall contractions with position in the grouping. The mean duration of first myopotentials was double that of fourth myopotentials (Table 2). This decrease in myopotential duration with position in the group over the first few bursts corresponded with the increase in call duration with position in the group. Overall, therefore, we found a negative relationship between the duration of individual bursts in the abdominal wall and the duration of the related vocalisation. High-repetition-rate bursts were separated by intervals ranging from 2.5 to 2.0 ms.

Costal part of the external abdominal oblique

Recordings of activity in the costal external oblique of *E. serotinus* during vocalisation were similar to those in the abdominal wall, but more variable and more prone to artefacts

Table 3. The association of single and double myopotentials of the lateral abdominal wall and of the external abdominal oblique (*pars costalis*) and vocalisations in *Eptesicus serotinus*

	Single	Double	Duration (ms)	N
Lateral abdominal wall				
With vocalisation	60	85	17.1±0.84	146
Without vocalisation	14	1	11.6±1.2	15
External abdominal oblique (<i>pars thoracis</i>)				
With vocalisation	35	70	19.4±1.19	109
Without vocalisation	2	0	13.4±3.8	2

In both cases, the occurrence of double bursts was more frequent when a vocalisation occurred, although in the case of the external oblique the sample size of breath cycles excluding vocalisation was small.

Total durations of activity (means ± S.E.M.) of the muscles in each breath cycle in the presence and absence of vocalisation are also given.

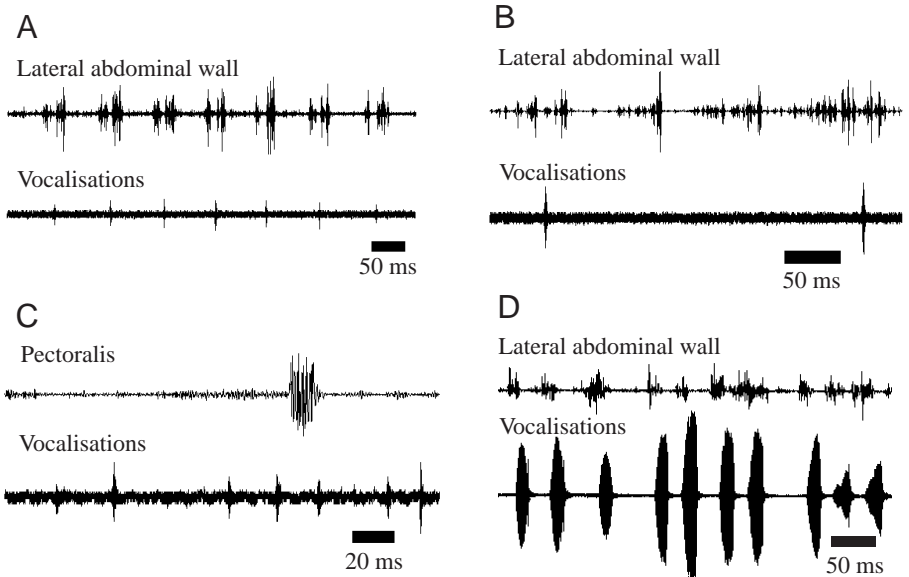


Fig. 4. Electromyograms recorded with respect to vocalisation from *Pipistrellus pygmaeus* (A–C) and *Pteronotus parnellii* (D) at rest. D is reproduced from Lancaster et al. (1995).

(Table 2) (Figs 3A–C, 6B). The pattern of muscular activity prior to and following vocalisation seen in the abdominal wall also occurred in the costal external oblique. Where recorded together, both the costal external oblique and the lateral abdominal wall were usually simultaneously active in relation to vocalisation, although costal external oblique onset consistently preceded activity in the abdominal wall by an average of 1.74 ± 0.45 ms ($N=107$). On average, bursts in the costal external oblique were slightly longer than associated bursts in the abdominal wall (Table 2). This difference was highly significant when comparisons were made between the costal external oblique contraction and the simultaneous contraction in the abdominal wall ($t=3.12$, $P=0.001$, d.f.=107). The mean difference in contraction duration of the two muscles matched almost exactly the mean difference in onset timing. Thus, on average, these muscles became active slightly asynchronously but ceased activity in synchrony.

The costal external obliques and abdominal wall were not successfully recorded simultaneously in *M. myotis*, but results of separate recordings were similar to those in *E. serotinus* (Fig. 5E). As with the abdominal wall, the number of contractions of the costal external oblique generally matched the number of vocalisations in each breath cycle. There was a similar pattern of significant decline in duration of activity in the costal external oblique in *M. myotis* with each successive burst in a group (one-way ANOVA; $F=7.77$, d.f.=368, $P=0.0001$). On average, the first contractions were over twice as long as the fourth contractions (Table 2). The first four contractions of the costal external oblique (mean across all four 5.22 ms) were significantly longer than those of the abdominal wall (mean 4.48 ms; $t=2.17$, d.f.=326, $P=0.031$).

Diaphragm (*pars costalis*)

In *E. serotinus*, there were generally two periods of activity

of the diaphragm in each breath cycle (20 of 21 breaths recorded). The durations of myopotentials were clearly bimodal, and each breath cycle included both a long- and a short-duration contraction (Fig. 3D,E). Short bursts averaged 4.93 ± 0.587 ms ($N=20$) in duration, and average onset occurred 4.62 ± 1.54 ms ($N=20$) before the vocalisation; thus, offset coincided almost exactly with the onset of vocalisation. The short contraction of the diaphragm appeared to occupy the gap between the offset of the activity of the abdominal wall and external oblique and the onset of the vocalisation. An additional short burst generally occurred if a second call followed (Fig. 3D). The onset of the long diaphragmatic myopotentials occurred on average 21.1 ± 2.85 ms ($N=21$) after the onset of calls, usually coinciding with cessation of activity in the external oblique and abdominal wall. Longer contractions were, thus, almost seven times longer than the short bursts of activity (Table 2).

Diaphragmatic activity in *M. myotis* followed the general pattern described above (Figs 5A,B, 6A) (Table 2). Greater variation in the duration and pattern of call groups contributed to greater variation in the timing of diaphragmatic activity. In general, diaphragmatic activity in *M. myotis* also included short and long bursts within each respiratory cycle. Although long bursts were obligatory in each cycle, short bursts occurred less frequently (22 of 66 cycles). Long-duration myopotentials from the diaphragm of *M. myotis* began after termination of vocalisation activity and were approximately 10 times longer than the short-duration bursts of activity. When short bursts occurred, onset did not precede or coincide with onset of the first vocalisation, as in *E. serotinus*. Rather, they generally occurred following groups of three or four vocalisations, resulting in a large variation in timing (Table 2). On average, short bursts were 3.55 ms in duration; on 10% of occasions (2 of 20 cycles), a second short burst was recorded. In some cases, short bursts occurred between the penultimate and ultimate calls and abdominal wall myopotentials of a group, suggesting that the bat replenished air briefly before finishing a call group (Fig. 5A,B).

Pectoralis

No activity was recorded from the pectoralis in stationary bats that appeared to relate to either vocalisation or respiration. To confirm the integrity of implants, pectoralis activity was recorded during brief tethered flights. These recordings showed pectoralis activity consistent with previously published

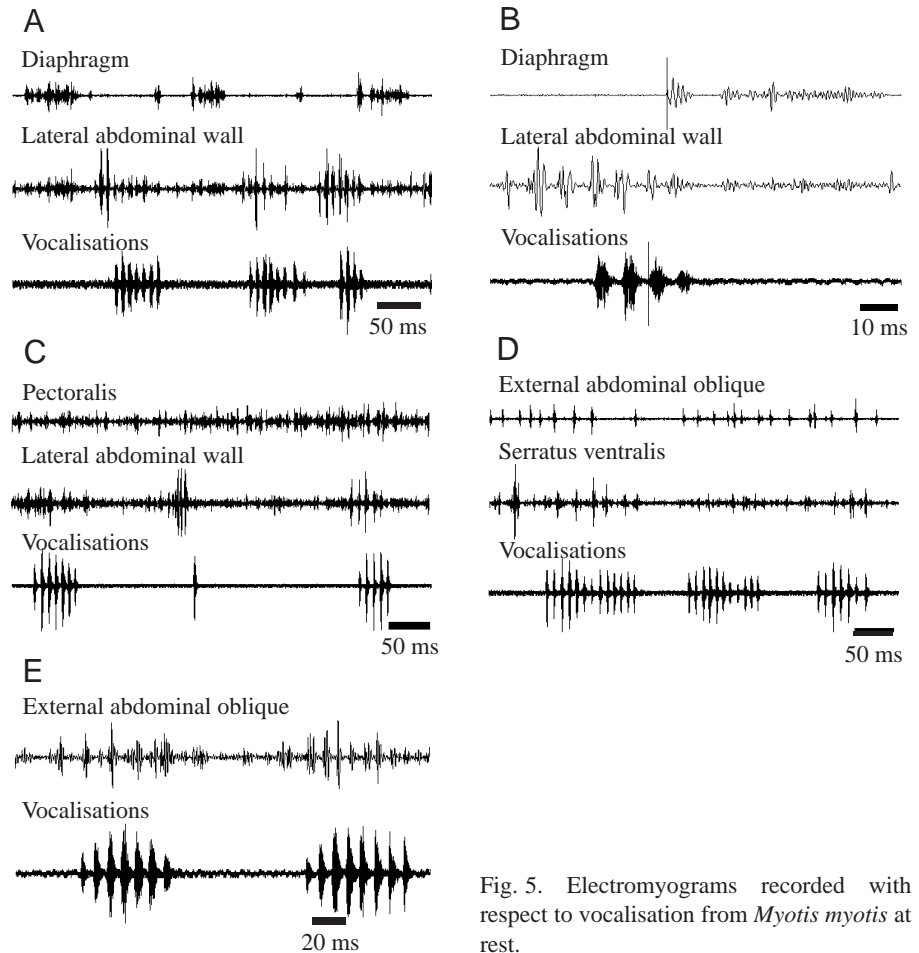


Fig. 5. Electromyograms recorded with respect to vocalisation from *Myotis myotis* at rest.

recordings (Lancaster et al., 1995; Hermanson and Altenbach, 1981, 1983, 1985). Fig. 4C depicts a recording of pectoralis activity in *Pipistrellus pygmaeus* hanging from the cage top and vocalising. In this instance, the bat dropped to the cage floor, resulting in a brief burst from the pectoralis as the bat landed, but none associated with the vocalisations.

Serratus ventralis

The serratus ventralis did not show powerful activity in relation to vocalisation or respiration in any of the three species of bat while at rest. As with the pectoralis, brief tethered flights confirmed the integrity of the implant, and activity diminished at landing (*E. serotinus*) (Fig. 3F). No relationship was evident between activity in the serratus ventralis and costal external oblique in *M. myotis*.

Respiratory muscle coordination

Fig. 7 is a histogram comparing the duty cycles of the muscles and vocalisation in each species. To calculate duty cycle over numerous respiratory cycles with variable numbers of calls and several recordings and individuals, we multiplied the mean duration of calls or myopotentials by the probability of their occurrence in our data sample. In both *E. serotinus* and *Pipistrellus pygmaeus*, the abdominal wall duty cycle exceeded

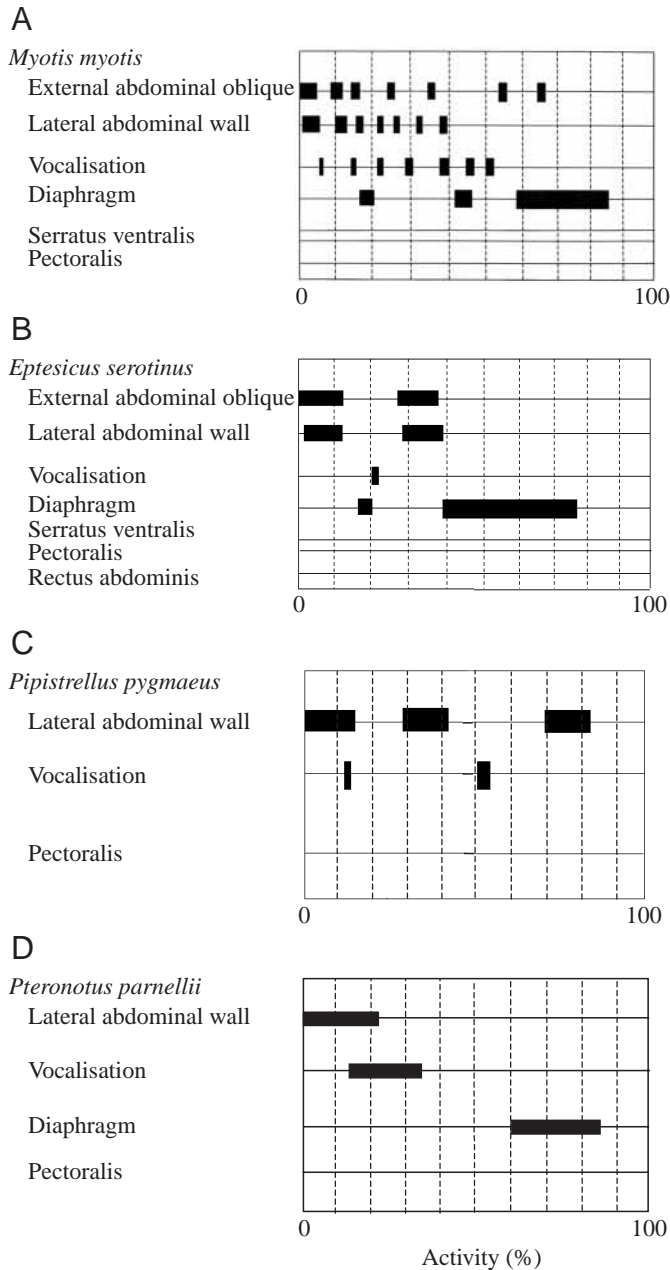


Fig. 6. Composite diagrams depicting the relative timing characteristics of respiratory and flight muscles activities and biosonar vocalisations at rest over a single respiratory period. (A) *Myotis myotis*; (B) *Eptesicus serotinus*; (C) *Pipistrellus pygmaeus*; (D) *Pteronotus parnellii*. Data for D are from Lancaster et al. (1995).

the vocalisation duty cycle threefold. The duty cycle of the costal external abdominal oblique was greater than that of the lateral abdominal wall in *E. serotinus*. In contrast, for a similar percentage of abdominal wall and external oblique activity, *M. myotis* doubled the proportion of the respiratory cycle in which vocalisation occurred. Diaphragmatic activity consumed a similar proportion of the respiratory cycle in the species in which it was recorded.

Discussion

Variations in the patterns and durations of muscle recruitment appear to be related to characteristics of echolocation calls. Here, we discuss variations in muscle activity with respect to vocal behaviour in bats with different styles of echolocation. We will consider how interspecific variation in muscle activity may relate to the style of echolocation, to the physical characteristics of calls and to the energetics of biosonar vocalisation.

Morphology

We found subtle morphological differences among species, but none that could reasonably account for the interspecific differences in biosonar behaviour and muscle recruitment that we recorded. The partition of the external abdominal oblique into separate costal and abdominal parts (Fig. 1) is an unusual detail that we have not previously seen in dissections or in published descriptions. This feature was consistent among the species we examined for this study and also in *Rhinolophus ferrumequinum*. However, in the megachiropteran *Rousettus aegyptiacus*, the thoracic and abdominal portions of the external oblique were continuous across the costal arch (W. C. Lancaster and J. R. Speakman, unpublished data), as is the case in most mammals. This feature allows the external abdominal oblique, pars thoracis and pars abdominalis, to function as two separate muscles. The thoracic part acts on the rib cage, and our data show that it is recruited in biosonar call production in parallel with the lateral abdominal wall. Its fascicles course roughly parallel to those of the transversus thoracis, which attach to the deep surface of the rib cage. The transversus thoracis has been identified as a primary muscle of expiration (De Troyer and Ninane, 1986; van Lunteren, 1991). Despite the difference in morphology, the external abdominal oblique pars thoracis appears to retain the expiratory function that is ascribed to all the muscles of the abdominal wall (Desmecht, 1996). Spanning from the costal margin to the pelvis, the abdominal part does not enter into the fabric of the abdominal aponeurosis and appears to retain the postural functions typical of this muscle in other mammals.

Vocalisation

All three species of vespertilionid were similar in their use of brief, frequency-modulated calls in contrast to the long, constant-frequency calls of *Pteronotus parnellii* (Fig. 2). Calls in *Myotis myotis* were shorter in duration in comparison with those of *Eptesicus serotinus* and *Pipistrellus pygmaeus*, but often consisted of groups of several calls in succession. Schnitzler (1968) reported similar behaviour in *M. myotis*, and this is characteristic of *Pteronotus parnellii* (Lancaster et al., 1995). The calls of these vespertilionids in flight under natural conditions are more variable and usually longer (Kalko and Schnitzler, 1993; Jensen and Miller, 1999; Ahlén and Baagøe, 1999).

Muscle activity in relation to vocalisation

Patterns of muscle recruitment during biosonar vocalisation

in the bats we studied resembled those in *Pteronotus parnellii* (Lancaster et al., 1995). Recordings from the external abdominal oblique could not be attributed with certainty to the single muscle because of the potential presence of both external and internal intercostals and the transversus thoracis near the recording site of the external oblique. However, the electrodes were in direct contact with the external oblique, whereas the other muscles mentioned were deeper. These other muscles could have contributed to the signals in these recordings that appear to be artefacts.

Recent studies on the function of respiratory musculature suggest that the topographic distribution of intercostal muscles may play a greater role in their function than fibre orientation (De Troyer et al., 1999b; Legrand and De Troyer, 1999). These studies found that the mechanical advantage of intercostal muscles varied between regions of the rib cage such that the action of both the external and internal intercostals over the dorsal-cranial region resulted in an inspiratory action. Similarly, both muscle layers contribute to expiratory function in the ventral-caudal region of the ribcage. Although the external intercostals in our specimens were small in the ventral-caudal region of the ribs, the costal portion of the external abdominal oblique in this region was well developed. It had a similar fascicular orientation to that of the external intercostal and of the transversus thoracis and was consistently active during vocalisation. Furthermore, the external abdominal oblique, acting in concert with the internal oblique and transversus abdominis, as in our recordings, increases intra-abdominal pressure and displaces the diaphragm cranially (Desmecht, 1996). We suggest that the muscles of the ventral-caudal rib cage have an expiratory effect on the thorax similar to the findings of De Troyer et al. (1999b). The onset of the myopotential in the external oblique prior to abdominal wall activity could serve to constrain the thorax against a paradoxical expansion, as suggested by Lancaster et al. (1995). Neither the flight muscles nor the rectus abdominis were recruited during biosonar vocalisation at rest.

Other species from which expiratory muscle activity was monitored during vocalisation showed variable behaviour. Single syllables in canary songs were consistently accompanied by abdominal muscle activity that usually overlapped with the onset of vocalisation (Hartley, 1990). Two syllables produced in a single expiratory phase were accompanied by two discrete bursts of muscle activity. However, during a lag of approximately 10 ms after the first burst, air-sac pressure continued to rise until the onset of the call (see figs 5 and 6 in Hartley, 1990). These delays resemble those we recorded in *E. serotinus* and *Pipistrellus pygmaeus* in the present study, and Hartley's data demonstrate that

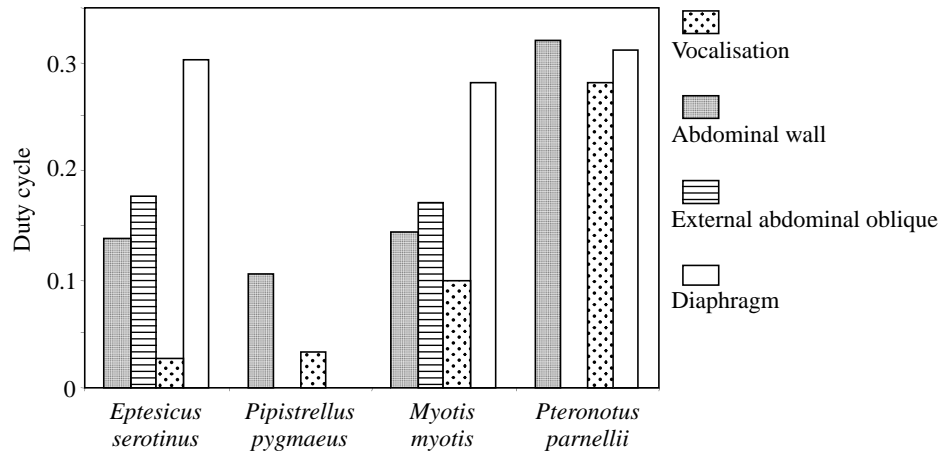


Fig. 7. Duty cycles of respiratory muscles in comparison with vocalisation at rest.

pressure can continue to rise after the cessation of muscle activity prior to vocalisation.

None of the vespertilionids showed the consistent overlap between myopotentials and calls seen in *Pteronotus parnellii*. Muscle activities in both the lateral abdominal wall and the costal external oblique in the vespertilionids consistently terminated prior to pulse onset. The only common instance of overlap came in *Pipistrellus pygmaeus*, in which calls sometimes overlapped the succeeding burst. This may relate to the differences in duration of calls. Being 4–8 times longer than calls we recorded from vespertilionids, the calls of *Pteronotus parnellii* consumed more of the respiratory cycle and required the generation of pressure for a longer period. Realisation of these levels of activity in *Pteronotus parnellii* may require overlap between muscle activity and calls. However, the overlap in *Pteronotus parnellii* is not merely a constraint of timing because it occurred irrespective of the rate of repetition or duty cycle and was not significantly different between flying and resting vocalisation (Lancaster et al., 1995).

A conspicuous feature of vespertilionid muscle activity was the common occurrence of bursts both prior to and succeeding calls. Succeeding calls occasionally occurred in *Pteronotus parnellii*, usually following periods of exertion, and appeared to be related to the rate of pulse repetition (Lancaster et al., 1995). In *E. serotinus* (Table 3) and *Pipistrellus pygmaeus*, bimodal myopotentials were more common than single bursts. These succeeding bursts were typically longer and often of higher amplitude than preceding bursts. This muscle activity was responsible in part for the dramatic difference in the duty cycle of calls compared with that of abdominal wall activity (Fig. 7). Similar to *Pteronotus parnellii*, most calls of *M. myotis* were associated with a single burst of abdominal wall activity; bursts succeeding calls were infrequent.

We did not examine expiratory muscle recruitment for expiration in the complete absence of vocalisation. Quiet expiration has traditionally been considered to be powered by the passive recoil of the chest wall. Forceful expiration is associated with muscle activity and may have occurred in recordings in which muscle activity followed a vocalisation

(e.g. Figs 3A, 4A). However, without a direct monitor of airflow, this cannot be substantiated. Recent studies show considerable variability among species in muscle recruitment during expiration and a strong influence of posture (van Lunteren, 1988). The inverted posture of bats would hypothetically facilitate quiet respiration by the pressure of the gut mass on the relaxing diaphragm. Data selected for analysis were recorded when bats were calm, to minimise complications, but even with an independent monitor of respiration, the difference between muscle recruitment for vocalisation *versus* respiration could not be separated.

Duty cycle offers an edifying means of comparing the muscular activity with its dynamic product. The averages of muscular and vocal duty cycles are presented for each species in Fig. 6, and these are summarised in Fig. 7. Duty cycle is particularly relevant to the comparison of vocalisation with respiratory muscle activities because the activities can be expressed as percentages of respiratory cycles. In this study, the duty cycle of *Pipistrellus pygmaeus* abdominal wall bursts exceeded that of vocalisation by threefold (Fig. 7). In *E. serotinus*, muscular duty cycle in both the abdominal wall and external oblique was five times longer than the vocal duty cycle. The duty cycle of abdominal wall contraction in *Pipistrellus pygmaeus* was slightly less than in *M. myotis*, but resulted in less than half as much vocalisation. Muscular duty cycles in *E. serotinus* were roughly equivalent to those in *M. myotis*, but again, the vocalisation output in *M. myotis* was three times greater. We interpret the dual periods of muscular activity, both preceding and succeeding bursts in *Pipistrellus pygmaeus* and *E. serotinus*, as work devoted more to expiration than to vocalisation. Clearly, the burst following a pulse served no function for vocalisation. Our data suggest that bats such as *E. serotinus* and *Pipistrellus pygmaeus* that appeared to be reluctant to produce biosonar vocalisations at rest devoted more muscular effort not only to vocalisation but also to respiration (but, as noted above, we have no direct evidence of this). None of the vespertilionids approached the efficiency of *Pteronotus parnellii*, in which the duty cycle of vocalisation was only 5% less than that of abdominal wall activity. In all species, diaphragmatic activity occupied approximately 30% of the respiratory cycle.

Although duty cycle provides a useful comparison of the relative timing of muscle recruitment for similar activities, it must not be extrapolated beyond the resolution of electromyographic data. Electromyography conveys no information about differences in the sizes of muscles among species, nor about the quantity of muscle that is recruited for the activity. These are important factors in biomechanical and energetic analysis that are beyond the scope of this study.

Diaphragmatic activity is similar in the three species in which it has been recorded. Activity consistently occurs between single calls or groups of pulses from all species in which it has been recorded. The grouping of calls that was evident in *Pteronotus parnellii* and was recorded here in *M. myotis* gave a clear clue as to the placement of inspiration. Short bursts of activity in the diaphragm were not reported in

Pteronotus parnellii (Lancaster et al., 1995). *E. serotinus* differed from *Pteronotus parnellii* in the consistent occurrence of short, low-amplitude myopotentials in the diaphragm prior to vocalisations. These are of interest because their relative timing places them in the interval between lateral abdominal wall bursts and calls (Fig. 6B). Their brief duration and occurrence soon after long diaphragmatic bursts argues against an inspiratory function, and they may function in a manner similar to the decrescendo bursts that follow inspiratory activity in other mammals (also known as postinspiratory inspiratory activity) (see van Lunteren, 1988). They differ from typical postinspiratory inspiratory bursts in their consistent association with vocalisations and occurrence 20–40 ms after an inspiratory myopotential (as opposed to directly following the inspiration). The traditional explanation of postinspiratory activity as a force to brake expiratory airflow and regulate end-expiratory lung volume (Remmers and Bartlett, 1977) does not fit the pattern we recorded. The occurrence of short diaphragmatic myopotentials in close succession to abdominal wall bursts may represent a reflex contraction resulting from stimulation of stretch receptors in the diaphragm. A similar effect was reported following mechanical vibration of the diaphragm in dogs (De Troyer et al., 1999a), but the functional value of this to echolocative vocalisation is unclear.

Diaphragmatic activity recorded from *M. myotis* only (Fig. 5A,B) resembled the mini-breaths reported in singing canaries by Hartley and Suthers (1989). Canaries consistently exhibited periods of inspiratory airflow during silent intervals of 15–90 ms during extended songs. We did not measure airflow, so cannot confirm a reversal of flow that would be necessary to verify a mini-breath. The diaphragmatic bursts we recorded came near the end of extended trains of pulses; at less than 10 ms in duration, they were much shorter than the mini-breaths in canaries. This activity was not observed in *E. serotinus*, but neither did this species use long trains of pulses at rest. *Pteronotus parnellii* would appear to be a likely candidate to use mini-breaths because of its frequent use of long trains of pulses, but this has never been reported. Fig. 5A,B shows that the brief diaphragmatic burst in *M. myotis* occurred between vocalisations, but also overlapped with activity of the lateral abdominal wall; two antagonistic muscles contracted simultaneously. These factors, in addition to the absence of this activity in the high-duty-cycle pulse trains of *Pteronotus parnellii*, suggest that these bursts are more likely to function to adjust pulmonary pressure or to adjust the precontractile lengths of fibres in antagonistic muscles. This activity does not resemble the postinspiratory inspiratory activity suggested for *E. serotinus*.

Muscle activity occurs during resting vocalisation to compress the ribcage, as well as the abdominal wall (Lancaster et al., 1995). The muscle suggested in that study, the transversus thoracis, was not recorded here. The transversus thoracis has been shown to be active during expiration (DeTroyer and Ninane, 1986), during vocalisation in humans (Estenne et al., 1990) and in the elevation of transplurial pressure in dogs (Legrand et al., 1998). However, the unusual

form of the thoracic part of the external abdominal oblique and its regular activity in relation to biosonar vocalisation are consistent with the hypothesis that significant energy may be required to constrain the rib cage against paradoxical expansion in the face of powerful contractions of the lateral abdominal wall. The force required is probably inversely proportional to the stiffness of the thoracic skeleton. Bats that use constant-frequency biosonar calls (such as the Rhinolophidae, Hipposideridae and *Pteronotus parnellii*) and that have a high propensity to vocalise while stationary (Roberts, 1972; Lancaster et al., 1995) tend to have more fused elements between and within the thoracic spine, ribs and sternum (Vaughan, 1970a; Lancaster et al., 1995, 2000). Vaughan (1970a) considered these morphological characteristics to be derived, and Jenkins (1970) considered that cranio-caudal expansions of the ribs serve to increase the stability of the thorax and vertebral column. The high-duty-cycle, constant-frequency style of echolocation is also considered to be a derived behaviour in comparison with frequency-modulated echolocation (Simmons, 1980; Fenton et al., 1995).

The energetic demands of intense biosonar vocalisation have moulded numerous aspects of the lives of bats, including style of foraging and the functional morphology of the axial skeleton. Vespertilionids use more muscular activity for vocalisation than *Pteronotus parnellii* and probably *Rhinolophus* sp. In comparison, vespertilionids recruit a greater mass of muscle, for a longer period per millisecond of call and show more variation in patterns of muscle recruitment. Bats that use constant-frequency echolocation decrease their dependence on muscular activity by relying on the passive stiffness of the rib cage and greater elasticity of the abdominal aponeurosis (Lancaster et al., 1995). The thoracic (non-flight) musculature is concomitantly reduced in mass, probably resulting in a slightly lower resting basal metabolic rate in addition to less energetically demanding vocalisation. The activity in the vespertilionids is more variable, relies on more muscles and uses a less derived abdominal aponeurosis in comparison with the bats that use constant-frequency echolocation.

Precise control of the timing and frequency content of vocalisation occurs in the larynx (Griffiths, 1983; Neuweiler, 2000). Although this is a vital component of the vocal system, the muscles are several orders of magnitude smaller than those of the thoracic and abdominal walls and we do not consider them to be a major contributor to the energetic costs of vocalisation.

The significance to bats of biosonar calls produced at rest varies among species. In bats that use the flycatcher mode of hunting, vocalisation at rest allows bats to scan the environment for passing prey and is, therefore, very adaptive. This behaviour has been documented in *Rhinolophus ferrumequinum* and other species of bat that use constant-frequency calls (Jones and Rayner, 1989; Neuweiler, 2000). Bats have complex social behaviour patterns and use vocalisations for intraspecific communication. In addition to

their functions in orientation, constant-frequency calls in *Pteronotus parnellii* also have communicative function (Kanwal et al., 1994). In addition, in a dark environment, biosonar calls may be as useful for orientation to a bat at rest as during flight (although clearly not as critical). Bats use a variety of audible social vocalisations, the production of which undoubtedly consumes energy. We have no data on the muscle recruitment patterns for these calls, so we can neither draw comparisons with biosonar vocalisation nor speculate on the energetic demands.

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References

- Ahlén, I. and Baagøe, H. J. (1999). Use of ultrasound detectors for bat studies in Europe: experiences from field identification, surveys and monitoring. *Acta Chiropt.* **1**, 137–150.
- Desmecht, D. (1996). Les muscles respiratoires des mammifères: Anatomie fonctionnelle. *Ann. Méd. Vét.* **140**, 179–192.
- De Troyer, A., Brunko, E., Leduc, D. and Jammes, Y. (1999a). Reflex inhibition of canine inspiratory intercostals by diaphragmatic tension receptors. *J. Physiol., Lond.* **514**, 255–263.
- De Troyer, A., Legrand, A. and Wilson, T. A. (1999b). Respiratory mechanical advantage of the canine external and internal intercostal muscles. *J. Physiol., Lond.* **518**, 283–289.
- De Troyer, A. and Ninane, V. (1986). Triangularis sterni: a primary muscle of breathing in the dog. *J. Appl. Physiol.* **60**, 14–21.
- Estenne, M., Zocchi, L., Ward, M. and Macklem, P. T. (1990). Chest wall motion and expiratory muscle use during phonation in normal humans. *J. Appl. Physiol.* **68**, 2075–2082.
- Fenton, M. B., Audet, D., Obrist, M. K. and Rydell, J. (1995). Signal strength, timing and self-deafening: the evolution of echolocation in bats. *Paleobiol.* **21**, 229–242.
- Fenton, M. B. and Bell, G. P. (1981). Recognition of species of insectivorous bats by their echolocation calls. *J. Mammal.* **62**, 233–243.
- Griffiths, T. A. (1983). Comparative laryngeal anatomy of the Big Brown bat, *Eptesicus fuscus* and the Mustached bat, *Pteronotus parnellii*. *Mammalia* **47**, 377–394.
- Hartley, R. S. (1990). Expiratory muscle activity during song production in the canary. *Respir. Physiol.* **81**, 177–188.
- Hartley, R. S. and Suthers, R. A. (1989). Airflow and pressure during canary song: direct evidence for minibreaths. *J. Comp. Physiol. A* **165**, 15–26.
- Hermanson, J. W. and Altenbach, J. S. (1981). Functional anatomy of the primary downstroke muscles in the pallid bat, *Antrozous pallidus*. *J. Mammal.* **62**, 795–800.
- Hermanson, J. W. and Altenbach, J. S. (1983). The functional anatomy of the shoulder of the pallid bat, *Antrozous pallidus*. *J. Mammal.* **64**, 62–75.
- Hermanson, J. W. and Altenbach, J. S. (1985). Functional anatomy of the shoulder and arm of the fruit-eating bat *Artibeus jamaicensis*. *J. Zool., Lond.* **205**, 157–177.
- Jenkins, F. A., Jr (1970). Anatomy and function of expanded ribs in certain edentates and primates. *J. Mammal.* **51**, 288–301.
- Jensen, M. E. and Miller, L. A. (1999). Echolocation signals of the bat *Eptesicus serotinus* recorded using a vertical microphone array: effect of flight altitude on searching signals. *Behav. Ecol. Sociobiol.* **47**, 60–69.

- Jones, G. and Rayner, J. M. V.** (1989). Foraging behaviour and echolocation of wild horseshoe bats *Rhinolophus ferrumequinum* and *R. hipposideros* (Chiroptera, Rhinolophidae). *Behav. Ecol. Sociobiol.* **25**, 183–191.
- Kalko, E. K. V. and Schnitzler, H.-U.** (1993). Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behav. Ecol. Sociobiol.* **33**, 415–428.
- Kanwal, J. S., Matsumura, S., Ohlemiller, K. and Suga, N.** (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoust. Soc. Am.* **96**, 1229–1254.
- Lancaster, W. C., Fenton, M. B. and Eger, J.** (2000). Morphology of the axial skeleton in relation to the style of biosonar. *Bat Res. News* **41**, 126.
- Lancaster, W. C. and Henson, O. W., Jr** (1995). Morphology of the abdominal wall in the bat *Pteronotus parnellii* (Microchiroptera: Mormoopidae): implications for biosonar vocalization. *J. Morphol.* **223**, 99–107.
- Lancaster, W. C., Henson, O. W., Jr and Keating, A. W.** (1995). Respiratory muscle activity in relation to vocalization in flying bats. *J. Exp. Biol.* **198**, 175–191.
- Lancaster, W. C., Keating, A. W. and Henson, O. W., Jr** (1992). Ultrasonic vocalizations of flying bats monitored by radiotelemetry. *J. Exp. Biol.* **173**, 43–58.
- Legrand, A. and De Troyer, A.** (1999). Spatial distribution of external intercostal activity in dogs. *J. Physiol., Lond.* **518**, 291–300.
- Legrand, A., Wilson, T. A. and De Troyer, A.** (1998). Rib cage muscle interaction in airway pressure generation. *J. Appl. Physiol.* **85**, 198–203.
- Link, A., Marimuthu, G. and Neuweiler, G.** (1986). Movement as a specific stimulus for prey catching behaviour in rhinolophid and hipposiderid bats. *J. Comp. Physiol. A* **159**, 403–413.
- Loeb, G. E. and Gans, C.** (1986). *Electromyography for Experimentalists*. Chicago: University of Chicago Press. 373pp.
- Neuweiler, G.** (2000). *The Biology of Bats*. Oxford: Oxford University Press. 310pp.
- Nomina Anatomica Veterinaria** (1983). Third edition. México City: International Committee on Veterinary Anatomical Nomenclature.
- Novick, A.** (1971). Echolocation in bats: some aspects of pulse design. *Am. Sci.* **59**, 198–209.
- Remmers, J. E. and Bartlett, D., Jr** (1977). Reflex control of expiratory airflow duration. *J. Appl. Physiol.* **42**, 80–87.
- Roberts, L. H.** (1972). Correlation of respiration and ultrasound production in rodents and bats. *J. Zool., Lond.* **168**, 439–449.
- Schnitzler, H.-U.** (1968). Die Ultraschall-Ortungslaute der Hufeisen-Fledermäuse (Chiroptera-Rhinolophidae) in verschiedenen Orientierungssituationen. *Z. Vergl. Physiol.* **57**, 376–408.
- Simmons, J. A.** (1980). Phylogenetic adaptations and the evolution of echolocation in bats. In *Proceedings of the Fifth International Bat Research Conference* (ed. D. E. Wilson and A. L. Gardner), pp. 267–278. Lubbock, TX: Texas Tech Press.
- Speakman, J. R., Anderson, M. E. and Racey, P. A.** (1989). The energy cost of echolocation in pipistrelle bats (*Pipistrellus pipistrellus*). *J. Comp. Physiol. A* **165**, 679–685.
- Speakman, J. R. and Racey, P. A.** (1991). No cost of echolocation for bats in flight. *Nature* **350**, 421–423.
- van Lunteren, E.** (1988). Respiratory muscle coordination. *J. Lab. Clin. Med.* **112**, 285–300.
- van Lunteren, E.** (1991). Contractile and endurance properties of the feline triangularis sterni muscle. *Respir. Physiol.* **85**, 279–287.
- Vaughan, T. A.** (1970a). The skeletal system. In *Biology of Bats*, vol. 1 (ed. W. A. Wimsatt), pp. 98–139. New York: Academic Press.
- Vaughan, T. A.** (1970b). The muscular system. In *The Biology of Bats*, vol. 1 (ed. W. A. Wimsatt), pp. 140–194. New York: Academic Press.
- Waters, D. A. and Jones, G.** (1995). Echolocation call structure and intensity in five species of insectivorous bats. *J. Exp. Biol.* **198**, 475–489.
- Winckler, G.** (1950). Contribution à l'étude des muscles larges de la paroi abdominale. *Arch. Anat. Hist. Embryol. Strasbourg* **33**, 159–228.
- Woodsworth, G. C., Bell, G. P. and Fenton, M. B.** (1981). Observations of the echolocation, feeding behaviour and habitat use of *Euderma maculatum* (Chiroptera: Vespertilionidae) in southcentral British Columbia. *Can. J. Zool.* **59**, 1099–1102.