

# No cost of echolocation for bats in flight

J. R. Speakman & P. A. Racey

Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, UK

ECHOLOCATION has evolved in relatively few animal species<sup>1</sup>. One constraint may be the high cost of producing pulses, the echoes of which can be detected over useful distances<sup>2</sup>. The energy cost of echolocation in a small (6 g) insectivorous bat, when hanging at rest, was recently measured at 0.067 Joules per pulse<sup>3</sup>, implying a mean cost for echolocation in flight of 9.5× basal metabolic rate (range 7 to 12×). Because flight is very costly<sup>4</sup>, whether the costs of echolocation and flying are additive is an important question. We measured the energy costs of flight in two species of small echolocating Microchiroptera using a novel combination of respirometry and doubly-labelled water<sup>5</sup>. Flight energy expenditure (adjusted for body mass) was not significantly different between echolocating bats and non-echolocating bats and birds. The low cost of echolocation for flying vertebrates may have been a significant factor favouring its evolution in these groups.

Two techniques have been used to estimate the energy costs of flight from respiratory gas exchange in bats and birds—respirometry and doubly-labelled water (DLW). Here we combined the two techniques by measuring the total energy expended by bats over an average period of 3.3 h using DLW. Approximately 0.5 h of this time was spent in free flight in a large room, whereas most of the rest was spent in a respirometry chamber. Assuming that non-flying time spent out of the chamber involved expenditure of energy at the same rate as when in the chamber, we estimated the non-flight costs and hence measured the flight costs by the difference.

Of 28 bats used, 25 were pipistrelles *Pipistrellus pipistrellus* (5.4 to 9.4 g) and three were brown long-eared bats *Plecotus auritus* (6.7 to 8.4 g). Bats were labelled with heavy oxygen and deuterium and placed in a respirometer for 90 min, after which blood samples were taken. The bats were then allowed to fly freely in a large darkened room (0.01 lux) for 60 min, followed by between 90 and 240 min of respirometry. A second blood sample was then taken. Five individuals flew for less than 600 s each, which was less than 10% of the time not measured by respirometry. Of the remainder, the time spent flying varied between 40 and 96% of the free-flight period (1,160 to 3,110 s). We failed to obtain sufficient blood at the second sampling for isotope analysis in six individuals.

For the five bats which flew for less than 600 s we evaluated energy expenditure during the entire time that they were out of the respirometry chambers. Because the time spent in flight by these bats was low, their estimated energy expenditure should be approximately the same as that measured at rest in the chambers. Therefore, these five estimates acted as controls. Flight cost estimates were made for 14 *P. pipistrellus* and 3 *P. auritus*.

The mean pre-flight energy expenditure over the 90 min prior to flight did not vary with time (Fig. 1a) and averaged 0.55 W (s.d. = 0.0318,  $n = 90$ ). Mean post-flight energy expenditure initially declined, from a level immediately after flight which was similar to the pre-flight level, to a minimum an hour later. Energy expenditure remained at this low level for a further 100 min. Only six individuals were monitored for longer than 160 min. These followed the same pattern as the other bats over the first 160 min and thereafter their energy expenditure increased to the pre-flight level (Fig. 1b). Over the first 160 min of the post-flight period the average energy expenditure was significantly lower ( $t = 3.69$ , d.f. = 248,  $P < 0.001$ ) than the energy expenditure over the 90 min pre-flight in the same individuals (mean post-flight expenditure 0.358 W, s.d. = 0.052,  $n = 160$ ). The difference between pre- and post-flight energy

expenditure in individual bats was not significantly correlated to either the time spent in flight, the energy expended in flight or body mass. Therefore the observed depression was probably not a compensatory mechanism to cover the energy cost of the flying.

The mean energy expenditure whilst out of the chamber for the five individuals which flew for less than 1,000 s was 0.56 W. This was evaluated by subtracting the energy expended within the chamber from the DLW estimate of total energy expenditure. During this time the bats spent >90% of their time at rest and their energy expenditure did not differ significantly from the mean pre-flight or immediate post-flight resting energy expenditure measured in the respirometry chamber.

For the 17 bats which flew for longer than 1,000 s there was no relationship between the flight energy expenditure and body mass (Fig. 2). The mean energy expenditure for the pipistrelles was 1.43 W (s.d. = 0.511,  $n = 14$ ) and for the long-eared bats was 1.3 W (s.d. = 0.554,  $n = 3$ ). These latter estimates do not differ significantly from the previous estimate for flight cost in *P. auritus*, evaluated by DLW alone, of 1.49 W (ref. 6).

The large individual variation in flight cost estimates (Fig. 2) was a consequence of precision error in the DLW technique. We have previously validated the DLW technique<sup>7</sup> by comparison with respirometry in these species and shown that the mean deviation of the DLW estimates from simultaneous respirometry was 13.5%. Because we subtracted the calculated resting costs measured by respirometry from the DLW estimates this error was amplified. The bats flew for an average of 1,754 s,

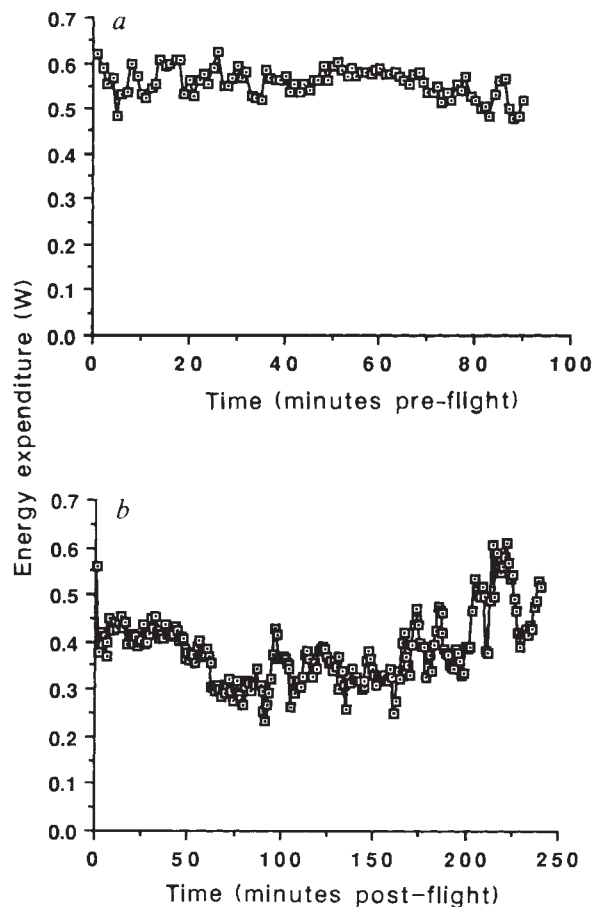


FIG. 1 a, Resting energy expenditure of bats averaged across 21 individuals measured over 90 min prior to flying for approximately half an hour and b, over 250 min following flight. During the post-flight phase the energy expenditure was depressed.

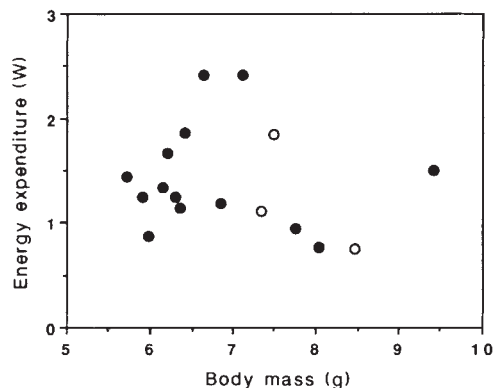


FIG. 2 Flight energy expenditure of small echolocating bats measured using a combination of DLW and respirometry, as a function of body mass. The closed symbols represent pipistrelle bats (*Pipistrellus pipistrellus*) and the open symbols brown long-eared bats (*Plecotus auritus*). Energy expended was measured using DLW over a period of 3.3 h, approximately 0.5 h of which was spent in flight and the majority of the remainder at rest in a respirometer. Carbon dioxide production was calculated using the Lifson and McClintock<sup>27</sup> equation with the pool size estimated from the dilution space of the <sup>18</sup>oxygen. This was converted to energy expenditure using the mean RQ of the pre- and post-flight respirometry sessions. The error in converting carbon dioxide production to energy expenditure using a known RQ is less than 1%<sup>28</sup>. We assume that the energy expenditure whilst out of the respirometry chamber, but not in flight, was at the same rate as the pre- and immediate post-flight resting rates. Therefore, the cost of flight was assessed from the difference between the DLW measure of total energy expenditure and the respirometry estimate of non-flying energy expenditure.

with a mean energy expenditure of 1.396 W. The rest time averaged 11,100 s, with a mean energy expenditure of 0.356 W and the expected amplification of variation was  $\times 2.38$ . The expected coefficient of variation in flight cost estimates due to analytical precision error was therefore 34.6%. The amplification of error masked any individual variation from biological sources. The use of DLW in combination with respirometry to estimate flight costs therefore provides no advantage over the use of DLW alone, when the time in flight is short relative to the time spent at rest, because it provides only a mean estimate across several individuals.

There is also an accuracy error in DLW, when compared with respirometry, which varies with the equation which is employed<sup>7</sup>. For the Lifson and McClintock equation, used to calculate CO<sub>2</sub> production here, we evaluated the mean accuracy error as +9.4% ( $n = 9$ )<sup>7</sup>. Because of amplification of error, this

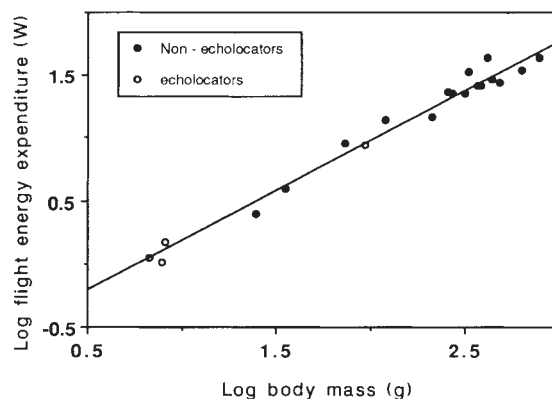
FIG. 3 Energy expended in flight of echolocating bats (open symbols) and non-echolocating birds and bats (closed symbols) plotted against body mass (g). The regression equations relating energy expenditure to body mass did not differ significantly between the two groups. For details of data used refer to Table 1. The best-fit equation for the combined data was  $\log_{10}$  energy expenditure (W) =  $-0.638 + 0.808 \log_{10}$  body mass (g);  $r^2 = 0.981$ .

TABLE 1 Estimates of energy expenditure for non-echolocating bats and birds, and for echolocating bats

Species	Mass (kg)	Energy expenditure (W)	<i>n</i>	Ref.	Technique*
Non-echolocating bats					
<i>Pteropus gouldii</i> (= <i>alecto</i> )	0.779	43.7	1	12	R
<i>Pteropus poliocephalus</i>	0.628	34.8	4	13, 14	R
<i>Hypsignathus monstrosus</i>	0.258	23.3	1	14	R
<i>Eidolon helvum</i>	0.315	22.4	1	14	R
Non-echolocating birds					
<i>Meliphaga virescens</i>	0.0243	2.46	?	15	R
<i>Melopsittacus undulatus</i>	0.035	3.93	1	16	R
<i>Sturnus vulgaris</i>	0.0728	9.15	6	17	R
<i>Falco spawerius</i>	0.120	13.8	3	18	R
<i>Falco tinnunculus</i>	0.213	14.6	6	19	R
<i>Corvus ossifragus</i>	0.275	22.8	2	20	R
<i>Larus atricilla</i>	0.370	26.2	2	21	R
<i>Columba livia</i>	0.384	25.9	4	22	D
<i>Columba livia</i>	0.442	29.5	6	23	R
<i>Columba livia</i>	0.412	43.7	6	24	D
<i>Columba livia</i>	0.330	34.0	6	4	R
<i>Corvus cryptoleucus</i>	0.480	27.8	2	25	R
Echolocating bats					
<i>Phyllostomas hastatus</i>	0.093	8.83	1	26	R
<i>Plecotus auritus</i>	0.008	1.35	3	6	D
<i>Pipistrellus pipistrellus</i>	0.0067	1.12	17	This study	R/D
<i>Plecotus auritus</i>	0.0077	1.02	3	This study	R/D

\* R refers to measurements made by respirometry and D to measurements made by doubly-labelled water. Measurements here were made by a novel combination of the two techniques. We treated as independent data values generated for different groups of animals in independent studies.

indicates that the estimates of flight cost were 23.3% too large and in our previous study<sup>6</sup>, 9.4% too large. We revised our current and previous<sup>6</sup> estimates in the light of the validation results<sup>7</sup>. All flight cost estimates for echolocating bats and non-echolocating bats and birds, using either respirometry alone, DLW alone, or DLW and respirometry combined are summarized in Table 1. We included only those birds for which estimates were made during sustained flights at the minimum power or maximum range speeds (for example, excluding hovering hummingbirds). We also excluded measures made on hirundines, swifts and terns, which have morphological adaptations to reduce flight costs<sup>8</sup> which are not found in the echolocating bats under study. Birds which spend a significant portion of the time gliding were also excluded. The gradients and intercepts of the relationships between  $\log_{10}$  flight energy expenditure and  $\log_{10}$  body mass, for echolocating bats and non-echolocating birds



and bats combined were not significantly different (Fig. 3; echolocators gradient = 0.7932, non-echolocators gradient = 0.8, analysis of covariance (ANCOVA) gradients  $F = 0.034$ ,  $p = 0.954$ , d.f. 1,20). Thus, it is clear that the estimates of flight cost in echolocating bats are not significantly greater than those for non-echolocating bats.

The absence of an energy cost for echolocation for flying animals may explain why echolocation systems are widespread in the Microchiroptera, but have evolved in very few terrestrial animals. Furthermore the high cost for terrestrial animals<sup>3</sup> may explain why those systems that have evolved in terrestrial mammals involve only very weak short-range pulses<sup>9</sup>. Two alternative hypotheses may also explain the paucity of, and low intensity of, terrestrial echolocation systems. First, the echolocation pulses may reveal the whereabouts of the emitter to potential prey and predators. Alternatively, in a complex and cluttered terrestrial environment the emitter may be confused by strong reflections from very close large objects. Our data strongly support the energy cost hypothesis but cannot rule out these alternative hypotheses in the evolution of echolocation.

The close link between flight and reduced costs for echolocation raises the issues of why echolocation has evolved so infrequently amongst the birds and Megachiroptera, and why, when it has evolved in these animals, it is primarily used for gross navigation rather than prey detection. We suggest that the paucity of echolocation systems amongst these groups reflects a phylogenetic constraint on the development of the processing capacity for complex echolocation signals in animals which are already evolutionarily committed to a visual system. Vision is clearly the dominant system amongst birds. Nocturnal birds have larger cortex areas devoted to processing olfactory stimuli than diurnal birds<sup>10</sup>. As total brain size remains unaffected this suggests there is a trade off in the processing capacity allocated to the various senses. This may have prevented any species from

making a complete evolutionary change from one system (visual) to another (echolocation) because the intermediate steps would be selectively inferior to either of the pure systems. The recent suggestion that the megachiroptera have a primate ancestry<sup>11</sup> is consistent with this interpretation because primates also have well-developed vision. □

Received 15 October 1990; accepted 12 February 1991.

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ACKNOWLEDGEMENTS. We thank P. I. Webb and A. M. Burnett for assistance, A. E. Fallick and T. Donnelly of the Scottish Universities Research and Reactor Centre, East Kilbride, for help and advice, and J. P. Hayes, G. C. Hays, M. B. Fenton and J. M. V. Rayner for constructive comments on the manuscript. This work was supported by the NERC.

## A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin $\mu$ chain gene

Daisuke Kitamura, Jürgen Roes, Ralf Kühn & Klaus Rajewsky\*

Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Cologne 41, Germany

OF the various classes of antibodies that B lymphocytes can produce, class M (IgM) is the first to be expressed on the membrane of the developing cells. Pre-B cells, the precursors of B-lymphocytes, produce the heavy chain of IgM ( $\mu$  chain), but not light chains<sup>1</sup>. Recent data suggest that pre-B cells express  $\mu$  chains on the membrane together with the 'surrogate' light chains  $\lambda 5$  and VpreB (refs 2–7). This complex could control pre-B-cell differentiation, in particular the rearrangement of the light-chain genes<sup>8</sup>. We have now assessed the importance of the membrane form of the  $\mu$  chain in B-cell development by generating mice lacking this chain. We disrupted one of the membrane exons of the gene encoding the  $\mu$ -chain constant region by gene targeting<sup>9</sup> in mouse embryonic stem cells<sup>10</sup>. From these cells we derived mice heterozygous or homozygous for the mutation. B-cell development in the heterozygous mice seemed to be normal, but in homozygous animals B cells were absent, their development already being arrested at the stage of pre-B-cell maturation.

\* To whom correspondence should be addressed.

The vector used for the disruption of one of the membrane exons ( $\mu M$ ) (Fig. 1A) contains 9 kilobases (kb) of genomic DNA spanning exons 1 and 2 of  $\mu M$  and the first three exons of the constant (C) region of the  $\delta$  gene. Close to the 5' boundary of the first exon of  $\mu M$  we introduced a translational stop codon and a *SalI* site into which a neomycin-resistance gene (*neo<sup>r</sup>*) cassette<sup>11</sup> was inserted. At the 3' end of the genomic sequence we placed the herpes simplex virus thymidine kinase gene to permit selection against random integration<sup>12</sup>.

Cells of the embryonic stem cell clone D3<sup>13</sup> were transfected with the linearized vector by electroporation and selected by G418 and gancyclovir on feeder layers of STO fibroblasts<sup>14</sup>. Surviving colonies were screened for homologous recombinants using the polymerase chain reaction (PCR) (see legend to Fig. 1). PCR-positive clones were expanded and their identification as homologous recombinants verified by Southern blotting (Fig. 1B). From  $3.4 \times 10^7$  transfected embryonic stem cells 1,870 colonies were resistant to G418 (determined by control plates), 230 were resistant to both G418 and gancyclovir and in six clones one of the two  $C\mu$  genes in the genome was modified by homologous recombination with the vector without random integration. Thus, the frequency of gene targeting was 1/38 G418<sup>r</sup>+GANC<sup>r</sup> (G418 and gancyclovir-resistant respectively) colonies, which corresponds to 1/312 G418<sup>r</sup> colonies or 1/5.7  $\times 10^6$  transfected cells.

The mutated clones were injected into blastocysts from C57BL/6 mice to generate chimaeric animals. As the D3 line is derived from an agouti mouse (strain 129/Sv), chimaeric mice could be identified by coat colour. Ten male chimaeras derived from four different mutated clones were mated to C57BL/6 females. One of these chimaeras, derived from clone 210, transmitted embryonic stem-derived chromosomes into the germ line as judged by the production of agouti offspring at a