several times through the viscera and foot; the hemolymph, after being strained through 250-μm nylon mesh, was added to an approximately equal volume of collection fluid consisting of 0.5*M* NaCl, 0.05*M* Hepes, 5 m*M* EGTA, 1 m*M* *p*-tosyl arginine methyl ester, Pepstatin A (3 μg/ml, Boehringer-Mannheim), and leupeptin (3 μg/ml, Boehringer-Mannheim) (*p*H 7.5). After the hemolymph preparation was centrifuged at 10,400*g* in a Sorvall centrifuge with an HB-4 rotor, fluid between the pellet and the floating lipid pad was removed and recentrifuged. The supernatant was then spun for 2 hours at 80,000*g* in an SW-39 rotor with an L-2 Beckman ultracentrifuge; the resulting pellet was used for gel electrophoresis.

9. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
10. Energy-dispersive spectroscopy was performed in a Philips 400 analytic transmission electron microscope fitted with an x-ray energy–dispersive spectrometer. Thin sections (60 to 80 nm) of auricular tissue that had been prepared for standard electron microscopy were mounted on aluminum grids. The analyses were done on clusters of hemocyanin molecules found within the hemocoelic spaces at 20-kV accelerating voltage, 15° specimen tilt, and a probe size of 100 nm for 300 live seconds. Ratios of energy peaks for copper K- and L-shell electrons to background were 4.8 and 2.2, respectively.
11. A. C. Redfield, *Biol. Rev.* **9**, 175 (1934).
12. R. C. Terwilliger, N. B. Terwilliger, E. Schabtach, *Comp. Biochem. Physiol.* **59**, 9 (1978).
13. C. M. Yonge, *Philos. Trans. R. Soc. London Ser. B.* **230**, 79 (1941).

14. Most of this research was done at the Friday Harbor Marine Laboratories, University of Washington. We thank S. A. Woodin, P. Illg, T. Schroeder, and D. Willows for critically reviewing the manuscript. Special thanks go to D. Henry for editorial assistance. This research was partially funded by DOE contract DE-ACOZ-77EVO4580. This is Northeastern University Marine Science Laboratory contribution No. 142.

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Flying Primates? Megabats Have the Advanced Pathway from Eye to Midbrain

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The pattern of connections between the retina and midbrain has been determined with electrophysiological and neuroanatomical methods in bats representing the two major subdivisions of the *Chiroptera*. Megachiropteran fruit bats (megabats), *Pteropus* spp., were found to have an advanced retinotectal pathway with a vertical hemidecussation of the kind previously found only in primates. In contrast, the microchiropteran bat *Macroderma gigas* has the "ancestral" or symplesiomorphous pattern of retinotectal connections so far found in all vertebrates except primates. In addition to linking primates and megachiropteran bats, these findings suggest that flight may have evolved twice among the mammals.

THE PATTERN OF CONNECTIONS between the retina and the midbrain superior colliculus (or tectum) distinguishes primates from all other mammals so far studied (*1*). In strepsirhine and haplorhine primates (*2*), the pattern of crossover of retinotectal fibers is like that of the retinothalamic fibers, with the result that the superior colliculus on one side of the brain subserves both eyes but only the opposite hemifield of visual space (*3*). In contrast, in all other vertebrate groups so far examined, the crossover pattern of retinotectal fibers differs from that of the retinothalamic fibers, with the result that the superior colliculus subserves the whole visual field of the opposite eye (*4–10*). Bats have not previously been studied in this regard, although the question is of some interest because some scholars have placed bats together with primates, dermopterans, and tree shrews in the superorder Archonta (*11*). Moreover, there are two distinct bat assemblages, one of which has an advanced visual organization with similarities to that of the primates. The latter are megabats, suborder *Megachiroptera*, a uniform Old World group of large, vegetarian bats reliant on their highly developed vision for foraging and obstacle avoidance (*12*). The microbats, suborder *Microchiroptera*, are, by contrast, diverse, worldwide, small, predominantly insectivorous bats, all of which use ultrasonic emissions for echolocation (*13*). I now report that fruit bats of the genus *Pteropus* have the advanced pattern of retinotectal fiber connections like that of primates. In contrast, the microbat, *Macroderma gigas*, has the plesiomorphous pattern of retinotectal projection found in most vertebrates. The findings lend support to the older classifications linking primates and bats, with the new qualification that this applies only to the *Megachiroptera*. A corollary of this phylogenetic hypothesis is that mammalian flight has evolved independently more than once (*14*).

The megabats used in this study were three grey-headed flying foxes, *Pteropus poliocephalus*, two black flying foxes, *Pteropus alecto*, and one little red flying fox, *Pteropus scapulatus*, taken from the wild near Brisbane, Australia, and maintained in an outdoor aviary. The microbats were two Australian ghost bats, *Macroderma gigas*, taken from a colony of 400 breeding females at Pine Creek, Northern Territory. *Macroderma* was chosen because, in comparison with most other microchiropterans, it has a relatively well-developed and experimentally tractable visual system, with large eyes and a temporal retinal area of increased ganglion cell density which "looks" forward like that found in *Pteropus* (Fig. 1). Two methods were used to determine the pattern of retinotectal fiber connections: electrophysiological and neuroanatomical. In the first, microelectrodes were used to record the visual responses of individual neurons in the superior colliculus of bats anesthetized with intramuscular injections of ketamine and xylazine (*15*). The locations of visual receptive fields were plotted with respect to the zero vertical meridian for each eye, the latter having been established from the projection of the ophthalmoscopically visible optic nerve head and data from retinal whole mounts giving the relation between the area of increased ganglion cell density and the nerve head (*16*). A check on this estimate was provided by binocular fields recorded in overlying visual cortex (*17*). For the neuroanatomical studies, injections of horseradish peroxidase (HRP), in some cases conjugated to wheat germ agglutinin (WGA-HRP), were made into the superficial layers of the superior colliculus receiving retinal input to enable retrograde labeling of retinal ganglion cells (*18*). The previous electrophysiological determination of the location of the superior colliculus was used to guide the placement of the HRP injections in three cases. In five other cases, the overlying visual cortex was removed by suction ablation to expose the superior colliculus so that injections could be directed visually to its whole retinal projection area. After survival times of 24 to 72 hours, the animals were killed with an overdose of anesthetic, perfused with fixative, and the brain and eyes removed. Retinal whole mounts were prepared to show the pattern of labeled retinotectal ganglion cells and the brain sectioned and reacted to verify the injection site (*19*).

All three *Pteropus* spp. examined electrophysiologically had the primate pattern of retinotopic organization in the superior colliculus. At the caudal edge of the superior colliculus, neurons had receptive fields in the far contralateral field, whereas at the rostral edge receptive fields were located close to the zero vertical meridian. No receptive fields were found more than 5° (the accuracy inherent in the method of plotting landmarks) into the ipsilateral hemifield. A

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majority of neurons could be driven independently by both eyes. In *Macroderma*, the topographical arrangement in the superior colliculus was with the lateral edge representing the lower visual field, the medial edge representing the upper field and the caudal edge representing the extreme contralateral periphery as in *Pteropus*, but responses at the rostral edge could be obtained from points in visual space which were as far as 30° across the zero vertical meridian in the ipsilateral visual field. Moreover, from none of the 30 sites studied in the rostral part of the colliculus of *Macroderma* could responses be elicited from the ipsilateral eye.

These electrophysiological differences between the retinotectal organization of the two groups of bats were graphically illustrated by the results of the retrograde labeling experiments. *Pteropus* showed a vertical decussation line passing through the specialized area of both retinas, with labeled retinotectal ganglion cells on one side of the line but not the other. Densities of labeled retinotectal ganglion cells were low (2 to 4×10^2 mm^{-2}) compared with the densities (2 to 4×10^3 mm^{-2}) of the total population of neurons in the retinal ganglion cell layer (*20*), although both ipsilateral and contralateral densities were comparable. The same pattern was observed in all five *Pteropus* (three species) studied. *Macroderma* showed no decussation line, with labeling across the whole extent of the contralateral retina and no labeled ganglion cells at all in the ipsilateral retina. The density of labeled cells reached 2.5×10^3 mm^{-2} in the contralateral retina, a significant fraction of the total population of neurons in the retinal ganglion cell layer, which peaked at 3.5×10^3 mm^{-2} (Fig. 1).

These results show that a major, representative genus of megabats has the advanced or synapomorphous pattern of retinotectal organization found in primates. In contrast, one of the most highly visual microchiropteran bats known does not have this pattern, but shares the primitive or plesiomorphous condition with other groups of mammals (and all other vertebrates). Apart from the musculoskeletal adaptations associated with the wing itself, there are no known synapomorphous characters which unequivocally link megabats and microbats (*21*), so a natural question arises as to the relative weighting to be given to these two conflicting synapomorphies: the wing and the hemidecussated retinotectal pathway. Is it more likely that flight evolved in parallel in two separate lines of mammals, one of them ancestral primates, or that megabats have evolved an advanced mode of retinotectal organization independently of primates (*22*)?

Separate origins for the wings of megabats and microbats are supported by the presence of a number of small, but consistent skeletal differences between them (*23*), the presence of other synapomorphies linking megabats to primates but not to microbats (*24*), the appearance of sustained flight in at least three separate nonmammalian lines (*25*), the numerous appearances of gliding flight with three separate "inventions" in the marsupials alone (*26*), and a fossil record indicating an origin for microbats more than 50 million years ago (*27*) compared with the recent fossil megachiropterans which have even been classified as primates (*28*). Separate origins for the advanced retinotectal organization in fruit bats and primates are not supported by the close identity between the complex details of the pathways involved in each group (*29*), nor by their absence in such highly visual and arboreal mammals as squirrels, cats, tree shrews, and phalangers (*5–10*). Serological evidence linking dermopterans and primates (*30*), the homologous structure and innervation of the patagium in dermopterans and megachiropterans (*11*), the present evidence linking primates and megachiropterans, plus the previous morphological evidence linking dermopterans, primates, and megachiropterans (*23*) all taken together, suggest that a fruitful line of future investigation will be the evolutionary relations of these three groups of mammals. In the meantime it

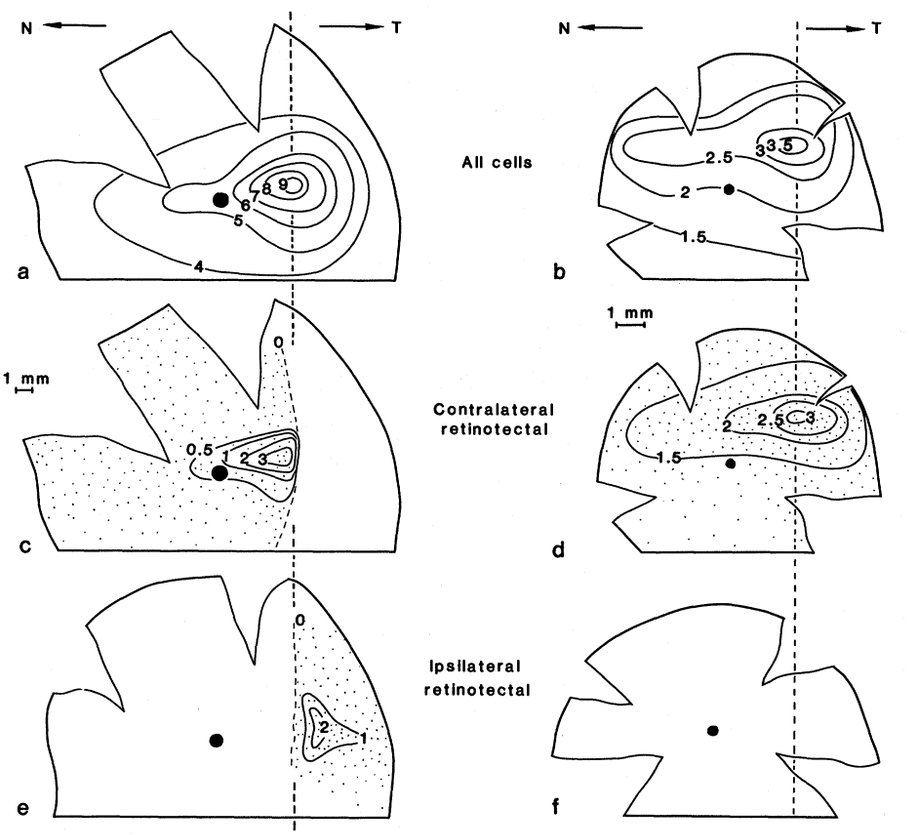

Fig. 1. Differing patterns of retinotectal organization in a megachiropteran bat, *Pteropus poliocephalus* (a, c, and e) and a microchiropteran bat, *Macroderma gigas* (b, d, and f). Retinal whole mounts are shown with contours of isodensity for cells in the retinal ganglion cell layer ($\times 10^3$ mm^{-2} in a, b, d, and f; $\times 10^2$ mm^{-2} in c and e), either stained with cresyl violet (*All cells*, a and b), or reacted to show the presence of HRP in ganglion cells contralateral (c and d) and ipsilateral (e and f) to the injection site in the right superior colliculus. Abbreviations: N, nasal; T, temporal. The ipsilateral retinas (from right eyes, e and f) have been mirror-reversed to facilitate comparison with the others. Stippling schematic indicates only the extent of the labeled retinal area. Both kinds of bats have a similar topography in the retinal ganglion cell layer (a and b) with a horizontal "streak" and a clearly defined area centralis in temporal retina subserving the region of frontal visual space. Despite the similarities in overall topography in the ganglion cell layer, there are dramatic differences in the topography of the retinotectal ganglion cells. In *Pteropus* retinotectal ganglion cells are found in relatively low density compared with the total in the ganglion cell layer, but both ipsilateral and contralateral retinas have comparable densities. There is a sharp decussation line, close to the vertical meridian of both ipsi- and contralateral retinas. No labeled ganglion cells could be detected (despite a long search at high power for any weakly labeled ones) temporal to the dotted line marked (0) in the contralateral retina (c) or nasal to the dotted line marked (0) in the ipsilateral retina (e). In *Macroderma*, retinotectal ganglion cells were found only contralateral to the injection site, with no indication of a decussation at the zero vertical meridian.

seems appropriate to propose that an early branch of the primate tree may have developed the power of flight long before the hominid branch even dreamed of it.

REFERENCES AND NOTES

1. J. M. Allman, *Prog. Physiol. Psychol.* 7, 1 (1977).
2. By this test and others, members of the *Menotyphla*, which includes the tree shrew, *Tupaia*, are now excluded from the primates, although they are the primates' closest "sister group" (*1, 9*).
3. Prosimian *Galago* [R. H. Lane, J. M. Allman, J. H. Kaas, F. M. Miezin, *Brain Res.* **60**, 335 (1973)]; new world primates, *Saimiri* [S. Kadoya, L. R. Wolin, L. C. Massopust, *J. Comp. Neurol.* **142**, 495 (1972)]; and *Aotis* [R. H. Lane *et al.*, *ibid.*]; old world monkey, *Macaca* [M. Cynader and N. Berman, *J. Neurophysiol.* **35**, 187 (1972)].
4. This is the "primitive" or plesiomorphous pattern [E. O. Wiley, *Phylogenetics* (Wiley, New York, 1981)].
5. Anurans [R. M. Gaze, *Q. J. Exp. Physiol.* **43**, 209 (1958)]; teleosts [H. Schwassman and L. Kruger, *J. Comp. Neurol.* **124**, 113 (1965)]; birds [H. Bravo and J. D. Pettigrew, *ibid.* **199**, 419 (1981)]; lizard [B. S. Stein and N. S. Gaither, *ibid.* **202**, 69 (1981)].
6. Rodents: rat [K. S. Lashley, *J. Comp. Neurol.* **59**, 341 (1934)], squirrel [W. C. Hall, J. H. Kaas, H. Killackey, I. T. Diamond, *J. Neurophysiol.* **34**, 437 (1971)], and ground squirrel [C. N. Woolsey, T. G. Carlton, J. H. Kaas, F. J. Earls, *Vision Res.* **11**, 115 (1971)].
7. Rabbit [A. Hughes, *Docum. Ophthalmol. (Den Haag)* **30**, 33 (1971)].
8. Cat [M. Straschill and K. P. Hoffman, *Brain Res.* **13**, 274 (1972)].
9. Opossum [C. Rocha-Miranda, R. Mendez-Otero, A. S. Ramoa, E. Volchan, L. G. Gawryszewski, in *Development of Visual Pathways in Mammals*, J. Stone, B. Dreher, D. Rapaport, Eds. (Liss, New York, 1984), pp. 179–198].
10. Tree shrew [J. H. Kaas, J. K. Harting, R. W. Guillery, *Brain Res.* **65**, 343 (1974)].
11. W. K. Gregory, *Bull. Am. Mus. Nat. Hist.* **27**, 332 (1910).
12. W. A. Wimsatt, *Biology of Bats* (Academic Press, New York, 1970).
13. M. B. Fenton, *Rev. Biol.* **59**, 33 (1984).
14. J. D. Smith, in *Biology of Bats of the New World Family Phyllostomatidae*, R. J. Baker, J. K. Jones, Jr., D. C. Carter, Eds. (Texas Tech Press, Lubbock, 1976), part 1, pp. 49–69; in *Major Patterns in Vertebrate Evolution*, M. K. Hecht, P. C. Goody, B. M. Hecht, Eds. (Plenum, New York, 1977), pp. 427–438; in *Proc. Fifth International Bat Research Conference*, D. E. Wilson and A. L. Gardner, Eds. (Texas Tech Press, Lubbock, 1980), pp. 233–244.
15. In two animals supplementation with Nembutal (pentobarbitone sodium) was used.
16. In *Pteropus* and *Macroderma* the vertical meridian was horizontally displaced from the blind spot approximately 18° and 20°, respectively. These values can be obtained from Fig. 1, given that in *Pteropus* 1 mm = 4.3° and in *Macroderma* 1 mm = 8° on the retina.
17. T. Nikara, P. O. Bishop, J. D. Pettigrew, *Exp. Brain Res.* **6**, 353 (1968).
18. In *Pteropus*, six to ten separate injections were made, totaling 1 to 1.5 μl of 20 percent HRP or 1 percent WGA-HRP solution, to involve the complete retinal projection area of the superior colliculus. In the case of WGA-HRP, which is colorless at the concentration used, fast-green dye was added to help gauge the degree of diffusion. In *Macroderma*, whose superior colliculus is only 2 mm across, two injections of 0.3 μl each were sufficient to involve the whole structure.
19. M. L. Cooper and J. D. Pettigrew, *J. Comp. Neurol.* **184**, 1 (1979).
20. Most of the remaining unlabeled retinal ganglion cells are retinothalamic ganglion cells projecting to the lateral geniculate nucleus (J. D. Pettigrew, M. L. Graydon, P. Giorgi, in preparation).
21. Most of the characters usually advanced to link megabats and microbats are associated with the flight adaptation [for example, characters 51 to 60 of M. J. Novacek, in *Macromolecular Sequences in Systematic and Evolutionary Biology*, M. Goodman, Ed. (Plenum, New York, 1982)], pp. 3–41. Other characters are contestable, having evolved in other unrelated mammalian orders (for example, fetal membrane characters 61 to 63, *ibid.*) or possibly representing plesiomorphous rather than synapomorphous characters (for example, 48 to 50, *ibid.*).
22. A third possibility is that the advanced mode of retinotectal organization arose first in megachiropteran bats, some of which later lost their powers of flight and gave rise to the primates. The extensive and fairly continuous fossil record of primates makes this scenario highly unlikely [M. Archer, in *Vertebrate Zoogeography and Evolution in Australasia*, M. Archer and G. Clayton, Eds. (Hesperian Press, Perth, 1983), pp. 949–993; F. Szalay and E. Delson, *Evolution and History of the Primates* (Academic Press, New York, 1979)].
23. J. D. Smith and A. Starrett, in *Biology of Bats of the New World Family* Phyllostomatidae, part 3, R. J. Baker, J. K. Jones, Jr., D. C. Carter, Eds. (Texas Tech Press, Lubbock, 1979), pp. 229–316; J. D. Pettigrew, K. S. Robson, K. I. McAnally, in preparation.
24. J. D. Smith and G. Madkour, in *Proceedings of the Fifth International Bat Research Conference*, D. E. Wilson and A. L. Gardner, Eds. (Texas Tech Press, Lubbock, 1980), pp. 347–365; J. E. Hill and J. D. Smith, *Bats: A Natural History* (British Museum, London, 1984).
25. K. Padian, *Paleobiology* **9**, 218 (1983); J. M. V. Rayner, *Symp. Zool. Soc. London* **48**, 137 (1981).
26. M. Archer, in Archer and Clayton [in (*22*), pp. 633–807].
27. M. Novacek, *Nature (London)* **315**, 140 (1985).
28. S. Hand, in Archer and Clayton [in (*22*), pp. 851–904]; A. Walker, *Nature (London)* **223**, 647 (1969).
29. There may be corollary developments in the telencephalic visual processing of movement by primates accompanying the advances in retinotectal organization, such as the MT (middle-temporal) visual cortical area found in primates, but in no other mammals so far studied (*1*). By the diagnostic criteria for MT that it be located in the temporal lobe and receive a major direct input from the primary visual cortex, this cortical area seems to be present in *Pteropus* [M. B. Calford *et al.*, *Nature (London)* **313**, 477 (1985)] but not in *Macroderma* (unpublished observations). The similarity of so many different and complex details of visual organization in primates and pteropids strengthens the argument against parallel appearance of the two systems.
30. J. E. Cronin and V. M. Sarich, in *Recent Advances in Primatology*, vol. 3, *Evolution*, D. J. Chivers and K. A. Joysey, Eds. (Academic Press, New York, 1978), pp. 287–288.

31. Supported by grants from the Australian Research Grants Scheme and the National Health and Medical Council of Australia. L. Wise and M. Calford helped with some of the electrophysiological recording experiments and provided critical comments on the manuscript. R. Collins provided expert technical assistance. Staff of the Conservation Commission of Northern Territory gave invaluable assistance in the collection of *Macroderma*, which were obtained under permit D85-5633.

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Structure of Ribosomal Subunits of *M. vannielii*: Ribosomal Morphology as a Phylogenetic Marker

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On the basis of ribosomal morphology, it has been proposed that the sulfur-metabolizing archaebacteria constitute a group (the eocytes) with a phylogenetic importance equal to that of the eubacteria, archaebacteria, and eukaryotes. It has been further proposed that eocytes should be given kingdom status. Ribosomal subunits from the methanogenic archaebacterium *Methanococcus vannielii* were examined by electron microscopy, and their structures were compared to those of other archaebacterial, eubacterial, and eukaryotic ribosomes. 30*S* subunits from *M. vannielii* showed the elongated contour and the one-third to two-thirds partition characteristic of such subunits. In addition, the angled asymmetric projections of those subunits showed a squarish base and a beak on the head. 50*S* subunits from *M. vannielii* were seen in both crown and kidney views. In crown views, the L1 protuberance was frequently pronounced and split; an incision below this protuberance and a protrusion at the base of the particle were also observed. Although previous studies suggested that certain of these structural features were found exclusively in ribosomes from sulfur-metabolizing archaebacteria, these new results indicate that such features also occur in ribosomes from a typical methanogenic archaebacterium and thus may not be reliable phylogenetic markers.

IT HAS BEEN PROPOSED THAT ANALYSIS of the structure of ribosomal subunits by electron microscopy is a rapid and accurate method for the classification of organisms (*1*). Five different ribosome structures have been described and have been assigned to four independent evolutionary lineages (*2–4*). We present here electron micrographs of ribosomal subunits from *Methanococcus vannielii* that are inconsistent with the assignment of one of these lineages (*2*).

The ribosomes used for this study were prepared as described (*5, 6*), and their activities in poly(U)-dependent polyphenylalanine synthesis were assessed (*6*). The sub-

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