other bronchial epithelial elements were eliminated or reduced by selecting peripheral areas of lung for homogenization. Electron micrographs of the pellet are shown in Fig. 2. The pellet was composed of "clouds" of plasma membrane and caveolae, many of which retain their characteristic morphology.

The plasma membrane–caveolae fractions were incubated at 37°C with (8-L-[^{14}C]phenylalanine)-angiotensin I in 2.0 ml of 50 m*M* tris-(hydroxymethyl)aminomethane-maleate buffer, *p*H 7.4, containing 100 m*M* KCl, 5 m*M* $MgCl_2$ and 100 m*M* Na_2HPO_4. (8-L-[^{14}C]phenylalanine)-angiotensin I (100 μc/μmole) was added in concentrations ranging from 0.4 to 4.0 μc/ml. Each reaction mixture contained plasma membrane (370 to 500 μg of protein per milliliter), derived from one-eighth to one-seventh of a lung. Reactions were stopped after 0.25, 5, 10, 15, 30, and 60 minutes of incubation by heating in a boiling water bath. Radioactive reaction products were identified by a combination of gel chromatography and paper electrophoresis (*1, 2*).

At any of the concentrations of angiotensin I used, conversion to angiotensin II was essentially complete (> 90 percent) within 15 seconds. Identification of angiotensin II was confirmed by bioassays with the rat colon and with mean arterial blood pressure preparations (*11*). The highest rate of conversion observed was 0.4 μmole per milligram of plasma membrane protein per minute. This rate is in excess of that likely to be required of lung in vivo, as angiotensin I probably occurs in concentrations of less than 50 pg/ml (~ 0.04 pmole/ml) in mixed venous blood (*7*). Angiotensin II, once formed, was not degraded in incubations of up to 60 minutes. Lower homologs were not observed. The reason for the failure of membrane fractions to form lower homologs is not known. Possibly, the peptidase enzymes capable of degrading angiotensin II are inhibited by the lead used for precipitation of inorganic phosphate.

Our data show that radioactive angiotensin I is metabolized during a single passage through the lungs. The volume of distribution of radioactivity does not exceed that of the intravascular space, and the mean transit time of radioactivity is no greater than that of blue dextran, a compound unlikely to leave the vascular lumen. Preparations of plasma membrane of lung are capable of converting angiotensin I to angiotensin II at rates sufficiently rapid to account for the conversion of angiotensin I to angiotensin II by intact lung. These points, taken together, are presented as strong support of the hypothesis that circulating angiotensin I is metabolized by enzymes of the luminal surface of pulmonary endothelial cells.

J. W. RYAN, UNA SMITH
R. S. NIEMEYER
Department of Medicine, University of Miami, and *Howard Hughes Medical Institute, Miami, Florida 33152*

References and Notes

1. J. W. Ryan, J. Roblero, J. M. Stewart, *Biochem. J.* **110**, 795 (1968); J. W. Ryan, J. M. Stewart, W. P. Leary, J. G. Ledingham, *ibid.* **120**, 221 (1970); J. W. Ryan, J. Roblero, J. M. Stewart, *Advan. Exp. Med. Biol.* **8**, 263 (1970); J. W. Ryan, J. Roblero, J. M. Stewart, W. P. Leary, *Chest* **59**, 8S (1971); U. Smith and J. W. Ryan, (Abstr.) *Pharmacol. Res. Commun.* **1**, 197 (1969); *Advan. Exp. Med. Biol.* **8**, 249 (1970); *Chest* **59**, 12S (1971).
2. J. W. Ryan, R. S. Niemeyer, D. W. Goodwin, U. Smith, J. M. Stewart, *Biochem. J.* **125**, 921 (1971).
3. J. W. Ryan and U. Smith, *Trans. Ass. Amer. Physicians* **84**, 297 (1971).
4. L. Binet and M. Burstein, *Presse Med.* **58**, 1201 (1950); S. H. Ferreira and J. R. Vane, *Brit. J. Pharmacol.* **30**, 417 (1967); K. K. F. Ng and J. R. Vane, *Nature* **216**, 762 (1967); P. Biron and C. Huggins, *Life Sci.* **7**, 965 (1968); J. R. Vane, *Brit. J. Pharmacol.* **35**, 209 (1969).
5. W. P. Leary and U. Smith, *Life Sci.* **9**, 1321 (1970).
6. F. P. Chinard, T. Ennis, M. F. Nolan, *Trans. Ass. Amer. Physicians* **75**, I-253 (1962).
7. G. Boyd, *Circ. Res.* **18**, I-105 (1970).
8. J. D. Barrett and M. P. Sambhi, *Res. Commun. Chem. Path. Pharmacol.* **2**, 128 (1971).
9. G. E. Sander and C. G. Huggins, *Nature New Biol.* **230**, 27 (1971).
10. J. W. Ryan and U. Smith, *Biochim. Biophys. Acta* **249**, 177 (1971).
11. D. Regoli and J. R. Vane, *Brit. J. Pharmacol.* **23**, 351 (1964); W. S. Peart, A. M. Lloyd, G. N. Thatcher, A. F. Lever, N. Payne, N. Stone, *Biochem. J.* **99**, 708 (1966).
12. Supported by grants from the U.S. Public Health Service (HE 12843 and 14360), and from the Council for Tobacco Research, New York.

26 October 1971; revised 10 December 1971 ■

Cochlear Microphonic Audiograms in the "Pure Tone" Bat Chilonycteris parnellii parnellii

Abstract. *Audiograms are sharply tuned to a frequency close to the second harmonic of the pulse. The tuning, which is much sharper than previously reported for any vertebrate receptor, provides a mechanism whereby the bat can effectively perceive echoes even during periods of pulse-echo overlap.*

Bats of the suborder Microchiroptera find their way about their environment and capture prey by echolocation (*1*). A number of species, often designated "CF" or "pure tone" bats, emit cries characterized by a long constant frequency (CF) component and brief beginning and terminal FM sweeps (*1–6*). It has been suggested that these two components have different functions, the FM sweeps being utilized for localization and ranging while the CF component is used for determining the relative velocity of the bat and its target (*4; 7–9*). Many species emit only brief FM pulses and they shorten their pulses as they approach a target to prevent pulse-echo overlap. However, in CF bats the pulse duration is so long that pulse-echo overlap is inevitable (*10*). How these bats extract information or even perceive echoes during periods of overlap has been a subject of considerable speculation (*3–5; 7–9*).

In the course of developing a technique for recording cochlear microphonic potentials from unanesthetized bats we obtained audiograms from nine *Chilonycteris parnellii parnellii* (Gray). These audiograms show the existence of highly specialized physiological properties in the cochlea. When coupled with detailed information on pulse design they offer a clear explanation as to how CF bats can effectively perceive echoes during periods of pulse-echo overlap.

Recording of cochlear microphonic potentials from unanesthetized animals was accomplished by intracranial implantation of tungsten electrodes near the cochlear aqueduct (*11*). With this technique high-amplitude potentials could be recorded with no interference to peripheral or central auditory structures. Audiograms were recorded from both anesthetized and awake animals (*12*) for periods of up to 3 weeks. During all experiments the bats were comfortably restrained with their heads held rigidly by a clamp attached to a keel of plastic cemented to the skull. The head was suspended in a free-field away from any objects, the wings were held away from the body and ears, and all surfaces behind the bats were lined with cotton to eliminate standing waves. The loudspeaker (*13*) was located 50 to 57 cm from the bat's ear and its orientation in the horizontal plane was adjusted

until high amplitude potentials were elicited by 45- to 60-khz tone bursts. Small changes in the position of the loudspeaker did not have a significant effect on the shape of the audiogram, and ear movements were not a problem as long as the animal was resting comfortably and loud sounds were not delivered to the ears.

All sound pressure level measurements were made with a calibrated ¼ inch condenser microphone (Bruël and Kjaer, model 4135) and sound level meter (Bruël and Kjaer, model 2604). Frequency measurements to within 10 hz were made with a precision frequency meter (General Radio, type 153-A). All experiments were conducted in a large, sound-proof, temperature-controlled chamber (Ray Proof, model R8293). Body temperature measurements could not be made in awake animals, but the room temperature was carefully controlled and the temperature of the ventral surface of the animal was continuously monitored and maintained at 36° to 37°C.

Cochlear microphonic thresholds were determined in two ways: (i) by recording the sound pressures at which responses to short tone bursts could no longer be distinguished above the noise level on the oscilloscope; (ii) by noting the intensity at which the response to a continuous tone first rose above the noise level of an RMS voltmeter (Bruël and Kjaer, model 2604). Both methods gave similar results.

As shown in Fig. 1, the audiogram of *C. parnellii* is sharply tuned. In each of the bats studied the audiogram had a unique "best frequency" in the 60.8- to 63.0-khz range (Fig. 2). The threshold on either side of the best frequency was found to increase very rapidly;

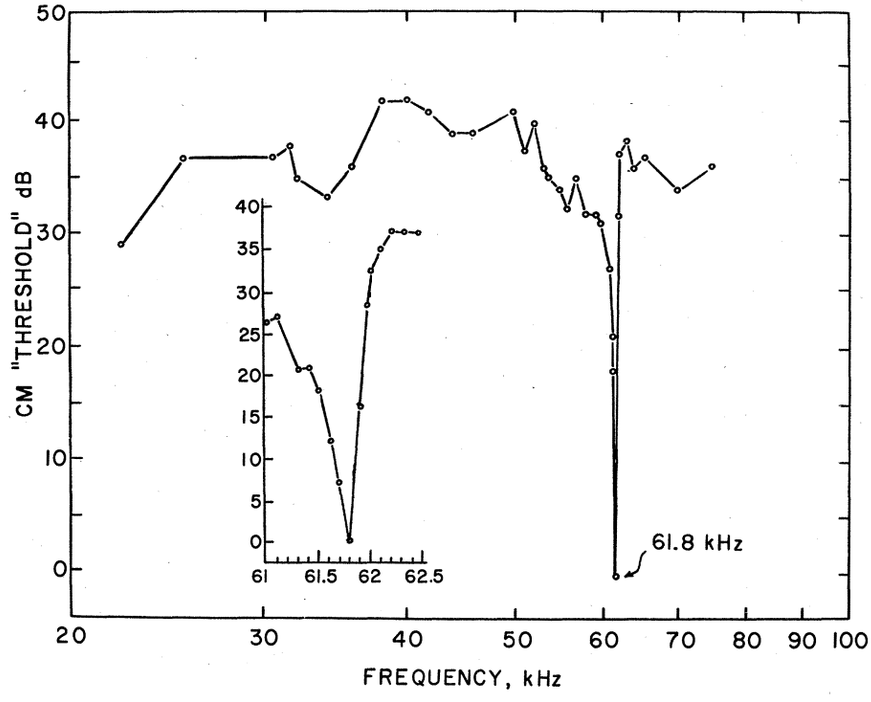
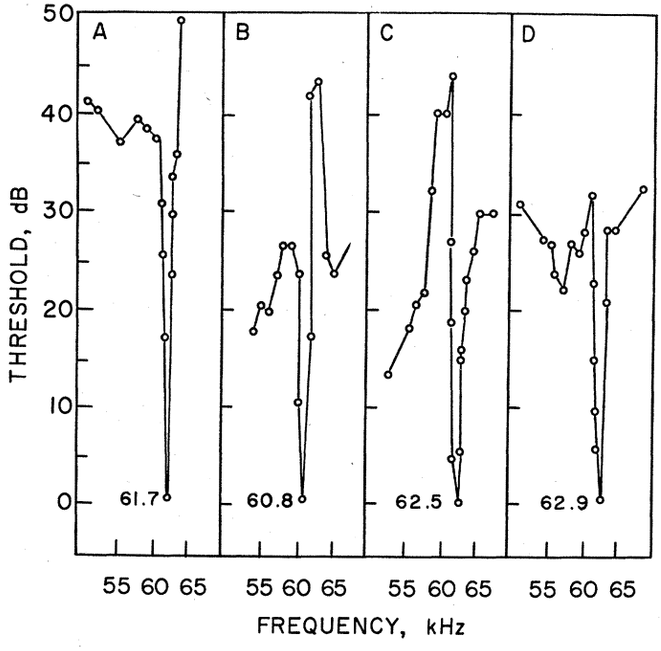
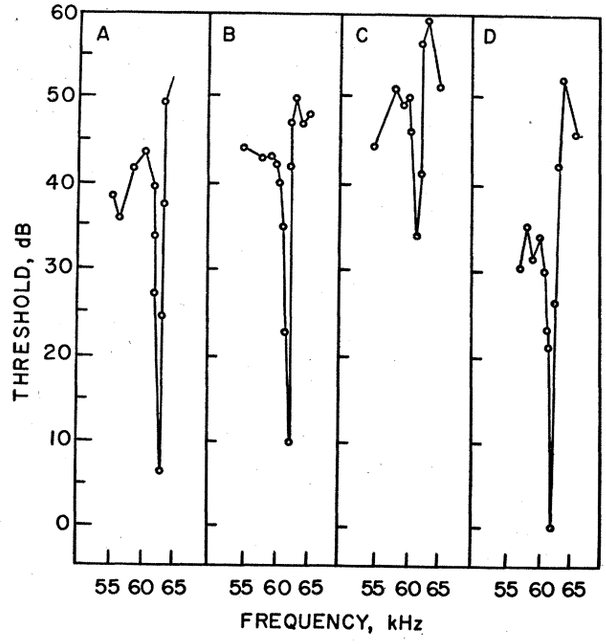


Fig. 1 (left). Cochlear microphonic audiogram from an awake bat. The threshold is expressed in decibels relative to the threshold at the best frequency (61.8 khz). The inset shows the sharply tuned portion of the same audiogram in greater detail.

Fig. 2 (bottom left). Cochlear microphonic audiograms from four awake bats. The threshold is expressed in decibels relative to the threshold of the best frequency for each bat. The exact values for the best frequencies are shown in each audiogram.

Fig. 3 (bottom right). Cochlear microphonic audiograms from one bat showing the depressive effect of 0.8 mg of sodium thiopental (0.08 mg per gram of body weight). The audiograms were determined on four successive days in the sequence shown. The bat was awake in A, B, and D but was anesthetized in C.





slopes of 150 db/khz were commonly encountered and in one animal the slope was 210 db/khz. The difference between the best frequency and adjacent insensitive portions of the audiometric curve ranged from 28 to 44 db on the low-frequency side and from 27 to 54 db on the high-frequency side (*14*).

Sharply tuned audiograms were encountered only in awake bats. In three anesthetized animals there was some tuning, but it was significantly different from that seen in the same bat after recovery from the anesthesia (Fig. 3).

The CF portion of the pulse was analyzed in several bats to determine the relation between the best frequency of the audiogram and the frequency of the CF pulse component (*15*). In all cases the frequency was significantly below the best frequency. The difference between the two frequencies averaged about 1500 hz.

The 1500-hz difference between the frequency of the CF component of the pulse and the best frequency of the bat's audiogram is equivalent to the amount of Doppler shift expected from the flight speed of *C. parnellii* (*10*). It appears that these bats, like the Rhinolophidae of Europe, emit a CF component to which their ears are relatively insensitive, but as long as there is any relative movement between a bat and its target the CF component of the echo can be Doppler shifted into a more sensitive portion of the hearing range (*4, 8*). In our most sharply tuned preparations the audiograms indicated that the cochlear receptor was up to 44 db more sensitive to Doppler-shifted echoes than to the frequencies of the emitted pulse. This, even without neural sharpening mechanisms (*5, 16*), could permit the system to function efficiently during periods of pulse-echo overlap.

Previous experiments have shown that bats in general, and CF bats in particular, have sharply tuned central auditory systems (*5, 9, 16, 17*). What is remarkable, however, is that the system is so exquisitely tuned as far peripherally as the receptor level. No cochlear microphonic audiogram in any vertebrate has yet been reported which even approaches the degree of frequency specificity in *Chilonycteris*.

The finding that anesthesia profoundly affects tuning at the receptor level was completely unexpected. Changes of this type have not been previously reported and are difficult to explain. However, they require that future experiments on the auditory system of CF bats, and perhaps other vertebrates, be carried out on unanesthetized, awake animals.

GEORGE POLLAK
O. W. HENSON, JR.
Department of Anatomy,
Yale University,
New Haven, Connecticut 06520
ALVIN NOVICK
Department of Biology, Yale University

References and Notes

1. D. R. Griffin, *Listening in the Dark* (Yale Univ. Press, New Haven, 1958).
2. A. Novick, *J. Mammal.* **44**, 44 (1963).
3. O. W. Henson, Jr., in *Animal Sonar Systems*, R. G. Busnel, Ed. (Laboratoire de Physiologie Acoustique, Jouy-en-Josas-78, France, 1967), vol. 2, p. 949; O. W. Henson, Jr., and M. M. Henson, AIBS–NASA Symposium on Animal Orientation and Navigation, Wallops Station, Va., 1970, in press.
4. H.-U. Schnitzler, *Z. Vergl. Physiol.* **68**, 25 (1970).
5. A. D. Grinnell, *ibid.*, p. 117.
6. The cruising pulses of *C. parnellii* are on the order of 20 to 25 msec in duration with a fundamental frequency of about 30 khz and a prominent second harmonic of approximately 60 khz (*2*). During the first millisecond of emission the frequency (second harmonic) often rises from about 56 to 60 khz and then stays constant (the constant frequency portion of the pulse); during the last 2 msec of emission the frequency sweeps downward to about 45 khz (the terminal FM portion of the pulse).
7. D. R. Griffin, *Symp. Zool. Soc. London* **7**, 61 (1962).
8. H.-U. Schnitzler, *Z. Vergl. Physiol.* **57**, 376 (1968); G. Neuweiler, *ibid.* **67**, 273 (1970).
9. O. W. Henson, Jr., in *Biology of Bats*, W. A. Wimsatt, Ed. (Academic Press, New York, 1970), vol. 2, p. 181.
10. A. Novick and J. R. Vaisnys, *Biol. Bull.* **127**, 478 (1964); A. Novick, *Amer. Sci.* **59**, 198 (1970).
11. O. W. Henson, Jr., and G. D. Pollak, *Physiol. Behav.*, in press.
12. Awake is defined as the ability to eat, drink, and emit pulses.
13. Designed after W. C. Kuhl, G. R. Schodder, and F. K. Schröder, *Acustica* **4**, 519 (1954).
14. The tuning was not influenced by the electrode position relative to the cochlear aqueduct. In one experiment, the audiogram was examined as the electrode was lowered through the brain in 0.5-mm increments without any apparent change in tuning. In several bats the electrode missed the aqueduct by 2 or 3 mm, but the audiograms were still sharply tuned. In contrast, the absolute thresholds were markedly affected by electrode placement. In the bats that had poor placements but sharp tuning the best frequency thresholds were 30 to 40 db higher than those that had properly positioned electrodes. Among the bats with good electrode placements, there was no more than ± 5-db difference in those thresholds.
15. Pulses were recorded with a condenser microphone during flight, while the bat was hanging on a wall, and while it was restrained as previously described. The output of the microphone was sent to a modified counter (General Radio, model 1192-B) which yielded a d-c signal directly proportional to the signal period in real time. The output of the pulse could thereby be measured to an accuracy of ± 100 hz.
16. N. Suga, *J. Physiol. London* **172**, 449 (1964).
17. A. D. Grinnell, *ibid.* **167**, 38 (1963).
18. This work was supported by PHS grant NB 7616-09.

30 September 1971

Nutritional and Environmental Interactions in the Behavioral Development of the Rat: Long-Term Effects

Abstract. *The behavioral effects of early malnutrition and early environmental isolation were observed in male rats. Dietary and environmental manipulations occurred during the first 7 weeks of life, after which followed a 10-week recovery period. On the basis of several different responses, it was found that the behavioral effects of early malnutrition were exaggerated by the environmental isolation. In most cases, the behavioral effects of early malnutrition were completely eliminated by supplying "additional stimulation" early in life. Two theoretical mechanisms are proposed to explain these findings.*

Malnutrition suffered early in the life of a child (*1*) or an experimental animal (*2*) has been shown to result in long-term behavioral abnormalities. In animals, the most characteristic change in adult behavior is an increase in emotional responsiveness (*3*). Other kinds of manipulations introduced very early in the life of animals also have long-term consequences upon adult behavior. Stimulation produced by daily handling of the neonatal rat has been reported to decrease adult emotionality (*4*). Moreover, early social or environmental isolation has been found to result in an increase in the emotional responsiveness of animals (*5*). The purpose of the study reported here was to compare the effects and possible interactions of early malnutrition and environmental conditions on various aspects of adult behavior in the rat.

All experimental manipulations occurred within the first 7 weeks of life. A 2 × 3 factorial design was used combining two levels of nutrition and three environmental situations. The nutritional treatment was as follows. Pregnant rats gave birth within our laboratory. The pups were reduced to eight per dam. All males were retained, and females were discarded. The dams were then