Middle Ear Muscle Contractions and Their Relation to pulse- and Echo-Evoked Potentials in the Bat (*Chilonycteris parnellii*)

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THE MUSTACHE BAT (*Chilonycteris parnellii*) emits orientation cries characterized by a long, 6- to 30-msec "pure tone" component and brief beginning and terminal FM sweeps (fig. 1). The long pulse durations make pulse-echo overlap inevitable under most conditions, and the question arises as to how the animals can effectively hear and analyze echoes that return during the emission of the intense outgoing pulse. In addition to the obvious masking problems during pulse-echo overlap, middle ear muscle contractions, if similar to those in other bats, should reduce hearing capacities during pulse emission and for several milliseconds thereafter (ref.1).

One method of recording the contraction characteristics of the middle ear muscles is to

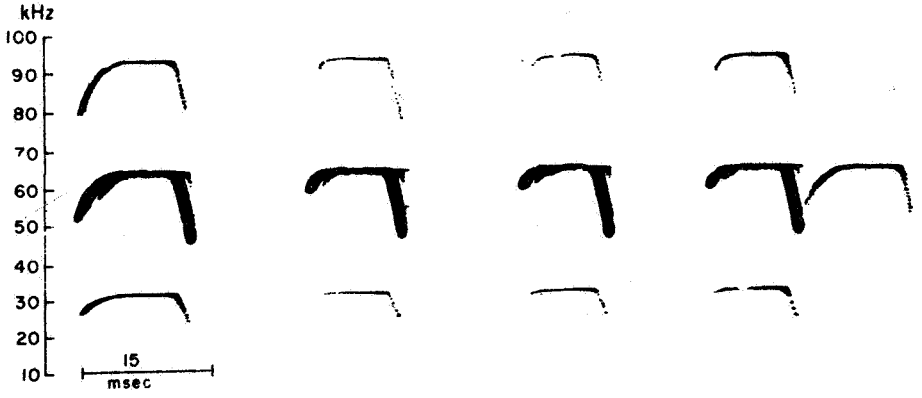

FIGURE 1. Sonograms showing frequency characteristics of pulses of *Chilonycteris parnellii parnellii*. Note strong 64-kHz second harmonic ("pure tone component") and the beginning and terminal FM components. Pulse number 1 on left shows unusually large initial FM component. Pulses 2, 3, and 4 are typical of those usually recorded from *Chilonycteris*.

place an animal in a pure tone, low frequency sound field and record the cochlear microphonic potentials evoked by the sound field. By observing the attenuation of the microphonic potentials, it is possible to record the exact time at which the muscles begin to contract, the speed of the contraction, the degree of contraction, and the time and rate of relaxation. This is the method which was successfully used in establishing the functional role of the muscles in the Mexican free-tailed bat (*Tadarida*) (refs. 1 and 2).

Recent application of this technique to *Chilonycteris* has yielded results which differ from those reported for *Tadarida* but which correlate with previous work dealing with pulse- and echo-evoked potentials in active *Chilonycteris*. In this report attention will be directed to:

(1) Characteristics of pulse- and echo-evoked potentials under various conditions

(2) Evidence of changes in hearing sensitivity during and after pulse emission

(3) The role of the middle ear muscles in bringing about these changes

MATERIALS AND METHODS

The bats used in this study were *Chilonycteris parnellii parnellii* (Gray) of the family Phyllostomatidae. The animals were captured in Jamaica and maintained in captivity on a diet of mealworms.

For recording cochlear microphonic potentials small stainless steel wires with the tips melted into a ball were chronically implanted on the round window of the cochlea. Uninsulated stainless steel insect pins (#00) placed within the substance of the inferior colliculi were used for recording evoked neural potentials. The techniques for recording cochlear potentials and brain potentials from unanesthetized, flying bats have been described previously (refs. 1 and 2).

Pulse- and echo-evoked potentials were studied under three different experimental conditions—in a small box; in a large chamber; and when flying within the chamber. The dimensions of the small box ($30 \times 30 \times 30$ cm) were such that the bat was always within 15 cm of one wall and most of the loud echoes should have returned to the bat's ear within several milliseconds. The large recording chamber measured approximately $3 \times 3 \times 3$ m and echo delays up to 17 msec were possible. The chamber was completely lined with orlon pile to reduce reverberating echoes; the floor of the chamber was covered with a carpet. The rug, the recording equipment, and the landing area probably produced the loudest echoes.

The exact position of the bat in the box or in the recording chamber at a given instant was not determined, but approximate positions were recorded by voice on one channel of a tape recorder (Precision Instrument Co., Model Pi–6100). The physiological potentials and the pulses emitted by the bats were recorded on other channels of the tape recorder.

Records of pulse- and echo-evoked neural potentials were obtained from seven animals; however, in only three of the seven did the animals appear to regain their preoperative state of health, as judged by strong flight and obstacle avoidance skills. The most extensive records were obtained from two animals with electrodes implanted in both inferior colliculi; potentials were recorded over periods of 6 and 9 days. In most cases the experiments were terminated when the electrode assembly became dislodged from the animal's head.

Chilonycteris did not tolerate extensive surgical exposure of the skull and round window; anchoring electrodes to only small exposed areas of the skull did not provide permanent preparations. Almost all of the cochlear microphonic potentials examined were

from two animals from which potentials were recorded for periods of 2 and 3 days after implantation of the electrodes. Although there was clear evidence of middle ear muscle contractions in all of the preparations, the amount of attenuation of the cochlear microphonic potentials was usually small in comparison with that observed in the Mexican free-tailed bat (*Tadarida*). The smaller attenuations (weaker contractions) in *Chilonycteris* may be attributed to the short postoperative recovery time and/or to differences in the development of the muscles. In *Tadarida*, which have very large muscles (ref. 3), strong muscle contractions were often not observed until 5 to 7 days after the operation (ref. 1), and the maximum amount of attenuation of the cochlear potentials ranged between 25 and 30 dB for the frequencies included in the emitted pulses. This amount of attenuation is sufficient to reduce an evoked potential of near maximum amplitude to a nondetectable response. In *Chilonycteris* even a 10 dB attenuation of the echo energy could account for the results observed in this study.

RESULTS

Pulse and Echo-Evoked Potentials

In all recording situations and in almost all records it was possible to identify three distinct fast wave potentials evoked by different components of the emitted pulse. These were most easily identified in the small box or in other confined spaces where there were no large echo-evoked potentials superimposed on the pulse-evoked potentials (fig. 2). The first fast wave potential was clearly associated with the beginning of each pulse. Its latency was on the order of 3.0 to 3.5 msec, and it represents the N_4 "on" response (ref. 4). Studies on *Myotis* have shown that this fast wave represents the lemniscal input

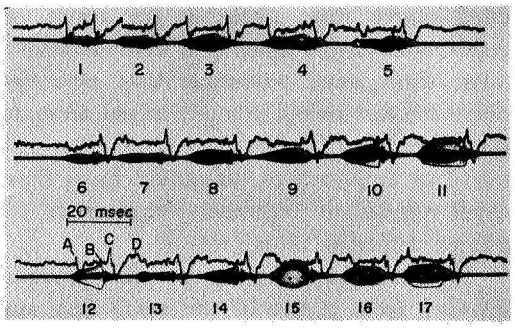

FIGURE 2. Typical examples of pulse-evoked potentials elicited by a bat's cry. Each upper trace shows evoked potential recorded from inferior colliculus; lower trace of each pair shows emitted pulse as detected by microphone placed several inches from the bat. Records were taken while the bat was in a small confined space so that echoes were reflected directly back into the ears. A—N_4 "on" response to the pulse's beginning, B—N_1 response probably to termination of the pure tone component or beginning of terminal FM sweep; C—sharp positive peak typical of "terminal" N_4 response; D—slow wave potential.

to the inferior colliculus (ref. 5). The second fast wave potential occurred within 1.0 msec of the termination of the pure tone component of the emitted pulse, or conversely, within 1.0 msec of the beginning of the terminal FM sweep. The amplitude of this potential was always small, and its latency was short; these characteristics suggest that it represents the N_1 (auditory nerve) potential. It is curious, however, that this potential was seldom seen in relation to the beginning of a pulse or in relation to echo-evoked potentials even when the echo-evoked N_4 potentials were large. The most consistent of all of the fast wave potentials was the "terminal" N_4 potential. This occurred 3 to 4 msec after the end of the pure tone component. The positive peak of the terminal N_4 response was not always sharp and distinct, but the negative going slope was always steep and easily recognizable. The fast wave peaks

noted above were probably the result of synchronous neural activity in many auditory units; slow wave potentials that typically follow the fast waves in anesthetized animals were seen (fig. 2), but they were often obscured by echo-evoked potentials. Such slow waves appear to represent the activity of inferior collicular units (ref. 5).

Typical examples of the evoked responses recorded from a bat in the small box are shown in figure 2. In these records the variable nature of the N_4 pulse "on" response is evident. The "on" responses are particularly large in relation to pulses 1, 3, and 4, but they are small and indistinct in relation to pulses 8, 9, 10, and 11. At fast pulse repetition rates the "on" response was often most pronounced in relation to the first pulse in a series, but no clear correlation could be found between the amplitude of the "on" response and the interpulse interval. Thus small "on" responses could not be correlated with poor recovery due to small interpulse intervals. In addition, no relationship could be found between the rise time of the emitted pulse and the amplitude of the "on" response. (For example, compare the rise times and the evoked responses shown in relation to pulses 9, 10, 11, and 12 in figure 2.) The amount of FM sweep showed no correlation with the amplitude of the pulse-evoked "on" response; large sweeps, similar to those shown for pulse 1 (fig. 1), sometimes produced smaller responses than did pulses showing little or no FM sweep.

The rise time and the amount of FM sweep at the beginning of a pulse are variable, especially when compared to the fall time and the amount of the terminal FM sweep. The more constant physical features at the end of the pulse are probably related to the consistent shape and amplitude of the pulse-evoked terminal N_4 potential. In almost all records there was a striking similarity in the pulse-evoked potentials from one pulse to the next. This is clearly evident in figures 2 and 3.

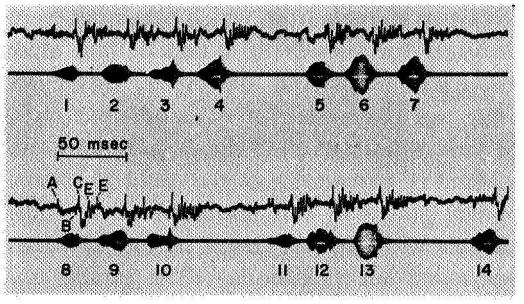

FIGURE 3. Pulse- and echo-evoked potentials recorded from inferior colliculus of *Chilonycteris*. Bat was on the ceiling of large recording chamber. A—N_4 "on" responses; B—N_1 response to "terminal" component of pulse; C—N_4 response to terminal component of pulse; E—echo-evoked potentials. Lower trace of each pair shows emitted pulses; pulses 1 to 7 are shown in upper record, and pulses 8 to 14 are shown in lower record.

The responses shown in figure 3 were recorded from a bat hanging on the wall of the large flight chamber. In relation to every pulse there is a distinct N_4 "on" response and an even more prominent N_4 terminal response; following the terminal N_4 pulse-evoked potential, there is a whole series of echo-evoked potentials. Although the exact origin of the echoes could not be established, there were a number of factors indicating that they were in fact echo-evoked potentials. First, such potentials were never recorded in the closed-space situations where all the echoes should have returned to the ear within a few milliseconds. Second, when the potentials did occur, their time of appearance after the pulse-evoked potential was consistent with the calculated echo delays for each situation. The most convincing evidence (ref. 2), however, is from the conclusive demonstration that the intervals between pulse- and echo-

evoked potentials continuously shortened in flying bats as they approached a known target (fig. 4).

There are three particularly important features shown in figure 3:

(1) All distinct echo-evoked potentials appear after pulse emission, and they occupy the intervals between pulses.

(2) There is a distinct neural silence between the N_4 "on" response and terminal N_4 response.

(3) The number of echo-evoked potentials is dependent on the interpulse interval. For example, there are four or five echo-evoked potentials which occur in the 8 msec interpulse interval between the end of pulse 11 and the beginning of pulse 12 (the fifth potential may be an "on" response to pulse 13). In the 12 msec interval between pulses 12 and 13 there are seven or eight echo-evoked potentials. After pulse 13, the last in the series, there are nine echo-evoked potentials. A large number of records similar to those shown in figure 3 have been analyzed. In almost all cases there were no echo-evoked "on" responses, and no distinct echo-evoked potentials of any kind could be identified consistently during pulse emission. After each pulse there was always a number of echo-evoked potentials. The only cases where echo-evoked potentials to a first pulse were recorded while a subsequent pulse was being emitted was when the second pulse was very faint.

Records obtained from flying bats were particularly interesting with respect to the amplitude of the echo-evoked potentials compared to pulse-evoked potentials. The echo-evoked potentials were as high as, or in many cases much higher in amplitude than, the pulse-evoked potentials (figs. 4 and 5). If the amplitude of the evoked potential reflects the number of units responding synchronously, it seems that the echoes are exciting more units

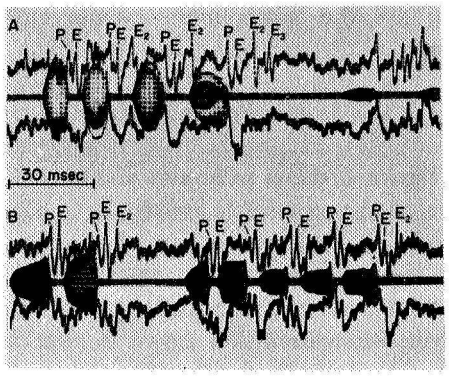

FIGURE 4. Neurophysiological evidence of focusing. Middle trace of each record shows emitted pulses; upper trace shows evoked potentials recorded from right colliculus; lower trace shows left collicular records. Echo-evoked potentials (E) recorded from right colliculus are masked if echoes return while subsequent pulse is being emitted. In A, echo-evoked response E_2 is large and distinct after pulses 2, 3, and 4 where it occurs during the interpulse interval. E_3 occurs only after pulse 4 where the interpulse interval is long. In B, echo-evoked potential E_2 is large and distinct after pulses 2 and 7. There is no distinct "E_2" potential following pulses 1, 3, and 6 where the interpulse intervals are short. In relation to pulses 4 and 5, there is a potential when the E_2 response should occur; these, however, also correspond to the appearance of the "on" responses for pulses 5 and 6.

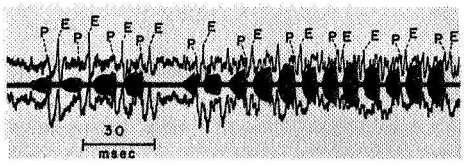

FIGURE 5. Pulse- and echo-evoked potentials recorded from right (upper trace) and left (lower trace) inferior colliculi of flying *Chilonycteris*. Middle trace shows pulses as detected by a microphone. Note the very high amplitude of the echo-evoked potentials (*E*) recorded from the right colliculus. As bat approaches target (a landing area), echo-evoked potentials occur closer to the pulse-evoked potentials (*P*), and their amplitude diminishes.

than are the much more intense emitted pulses.

The Middle Ear Muscles

Figure 6 shows examples of the cochlear microphonic potentials evoked by a 12-kHz sound field and attenuation of these potentials by contractions of the middle ear muscles. The most consistent type of microphonic attenuations are shown in relation to pulses 1, 5, 7, 8, 9, 11, 20, and 21. The muscles began to contract almost simultaneously with the beginning of each pulse, reached a maximum state of contraction prior to the terminal FM sweep, relaxed over the duration of the FM sweep, and completed the relaxation phase within 2 or 3 msec after the termination of the pulse. The attenuation shown in relation to pulse 3 is one of the strongest recorded, and in this case the contractions started about 4 msec prior to pulse emission. When faint pulses were emitted (e.g., pulses 4, 6, 10, 12, 13, 24, and 25), there was little or no evidence that the middle ear muscles contracted. During the terminal phase of echolocation or in other situations where *Chilonycteris* emitted pulses at very fast repetition rates (pulses 14 to 19, fig. 6), the muscles appeared to remain tonically contracted while the entire series of pulses was emitted.

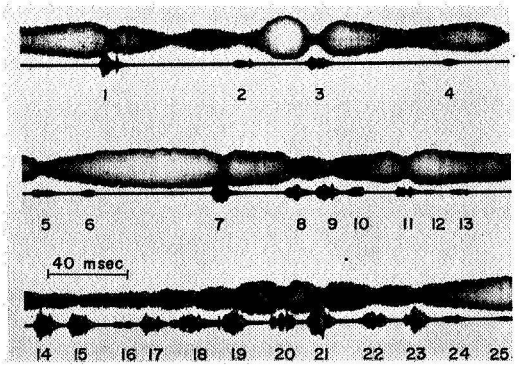

FIGURE 6. Middle ear muscle contractions in relation to pulse emission. Upper trace of each record shows 12-kHz sound field-evoked cochlear microphonic potentials, and lower trace shows emitted pulses detected by a microphone.

DISCUSSION

There appears to be one specific part of the bat's cry and of each returning echo that causes the synchronous firing of many auditory units and gives rise to the sharp, very distinct "terminal" N_4 potential. It was originally thought that this potential was evoked by the beginning of the FM sweep and thus represented an "on" response (ref. 2). Grinnell (ref. 6), however, has pointed out that it could be evoked by the termination of the pure tone component of the pulse. Both of these events occur simultaneously. Another possibility is that many units are excited at the instant the FM component sweeps through a specific frequency to which the ear is sharply tuned (ref. 7).[1] In *Chilonycteris* it has been shown that hearing is very sharply tuned over a narrow 1- or 2-kHz band which corresponds in frequency to the second harmonic of the emitted pulse (ref. 6).

Regardless of the origin of the potential, it can be demonstrated that there is a linear relationship between stimulus intensity and the amplitude of the evoked potentials at low and moderate sound pressure levels (ref. 2). At high sound pressures the linearity does not always exist, and a very loud pulse may produce a smaller response than a fainter pulse. Thus it is not correct to conclude that changes in the amplitude of the N_4 potential represent changes in sensitivity when dealing

[1] HENSON, O. W., JR.: The Ear and Audition. *In*: Biology of Bats. Vol. II. W. A. Wimsatt, ed., Academic Press (in press).

with loud signals. In the present study sensitivity changes were assessed on the basis of the presence or absence of echo-evoked potentials and not simply on minor amplitude changes. Under the "open space" experimental conditions, it seems unlikely that any of the echoes were extremely loud. In interpreting the results, it has been assumed that the fast wave N_4 potential indicates a synchronous firing of many units and that the appearance of the fast wave potential in relation to the echo is an important part of the echo detection system. Failure to record a potential not only indicates a lack of, or marked decrease in, synchronous firing, but it also suggests a reduction in sensitivity. The proposed reduction in sensitivity should not be equated with a lack of perception.

There are several findings in the evoked potential records which clearly indicate a decrease in sensitivity during pulse emission. "On" response echo-evoked potentials were seldom observed, and under the experimental conditions the beginning of the echoes should have returned while the pulse was being emitted. Also, there was usually little or no evidence of any type of potential while the pulse pure tone was being emitted (figs. 2 and 3). Most convincing was the finding that a specific "terminal" N_4 echo-evoked potential was large if it returned during the interpulse interval but would disappear if the echo returned while the next pulse was being emitted. Many records were obtained in which an echo-evoked potential would appear, disappear, and re-appear depending on whether or not the echo was overlapping the pulse (figs. 3 and 5).

It is important to note that the sensitivity changes indicated by the evoked potentials are exactly what one would expect knowing the contracting characteristics of the middle ear muscles. If muscle contractions start before or simultaneously with the beginning of the emitted pulse and reach a maximum state of contraction prior to the terminal FM sweep, then the energy at the beginning of an echo should be less efficiently transferred to the cochlear fluids than the energy at the beginning of the pulse. On the other hand, since middle ear muscle relaxations begin about the time of the terminal FM sweep and are completed within a few milliseconds after the end of the pulse, the FM echo energy should be transferred more efficiently than the FM pulse energy. This is at least a partial explanation for the very large echo-evoked potentials seen in flying bats.

It is important to note that under all conditions the first high amplitude echo-evoked potential to occur after each pulse was seen only when the pulse-echo delay was more than 3 msec; also important is the fact that the echo-evoked potentials became smaller in amplitude as the pulse-echo delay decreased (fig. 4). These observations can be correlated with the degree of muscle relaxation at specific points in time after the beginning of the terminal FM sweep.

The crucial experiment of denervating or ablating the middle ear muscles and then examining the evoked potentials has not been accomplished; it seems unlikely that the animals would survive such an extensive surgical procedure or that they would continue to emit intense pulses after such an operation. As noted above, however, our records indicate that the middle ear muscles do not contract when faint pulses are emitted (fig. 6), and in this connection it should be recalled that distinct echo-evoked potentials were recorded during pulse emission only when the second pulse was much fainter than the first.

In anesthetized *Chilonycteris* the N_4 sensitivity to the second of two identical signals is progressively reduced as the interstimulus interval is decreased (ref. 2). This may contribute to the small amplitude of the echo-

evoked potentials immediately following a pulse, but there are several points which suggest that the muscle contractions are more important in bringing about the amplitude reduction than are the recovery periods. Middle ear muscle relaxation during the terminal FM sweep in *Chilonycteris* is similar to middle ear muscle relaxation in *Tadarida*, and it has been observed that the cochlear microphonic potentials evoked by echoes in flying *Tadarida* become progressively smaller in amplitude as the animal approaches an echo source (ref. 2). Since the cochlear microphonic potential does not have a recovery period, the changes in amplitude can be attributed to incomplete relaxation of the middle ear muscles.

A second factor suggesting that middle ear muscle contractions are more significant than recovery periods in producing the results is that high amplitude evoked potentials were often recorded from *Chilonycteris* when the interval between the two echo-evoked potentials was only 1 or 2 msec (fig. 3). Although the relative intensities of the different echoes are not known, the responsiveness of the system to successive sounds appears to be considerably greater than has been observed in anesthetized preparations. In anesthetized *Chilonycteris* the time required for the evoked responses to show a 100-percent recovery for short trains of 90-dB (re-0.0002 dyne/cm^2) pulses was usually more than 10 msec, and in most cases no responses were observed when the interstimulus interval was less than 3 or 4 msec (ref. 2). Thus the system seems considerably more responsive to successive sounds in the unanesthetized than in the anesthetized animal at times when the middle ear muscles are relaxed.

There are a number of factors other than middle ear muscle tension that can control auditory input to some degree; these include ear position (refs. 2 and 8 to 11), auditory canal closure (ref. 11), and auditory feed-back systems within the CNS (ref. 12). By using relatively low frequencies for the pure tone sound field, the effect of the external ears and ear closure was minimized. From direct observation of these structures in *Chilonycteris* it is our opinion that they were not operating in such a way as to attenuate the sound field-evoked microphonic potentials in the brief time before, during, and after pulse emission. Furthermore, in both the round window implants and the inferior collicular implants, the mobility of the external ears was greatly reduced by the surgical procedure required to expose these structures; in all cases the extrensic ear muscles had to be severed from their origins on the back and top of the skull.

The possibility that efferent systems and/or pulse-echo interaction in the form of masking are contributing to the results of this study cannot be discounted. However, such factors, if present, appear to be operating at the same time and in much the same way as the middle ear muscles. In any case, sensitivity to echoes is at its best in the interval between pulses. In the past, the author has advocated that bats may be able to control the "echolocative depth of field" by controlling the duration of the interpulse interval (refs. 1 and 2). This idea was based on the known activity of the middle ear muscles in *Tadarida* and on the echo-evoked potentials in *Chilonycteris*. With the available data on evoked potentials and middle ear muscle activity for the same species, the assumption seems even more plausible than before.

SUMMARY

The orientation cries of the bat (*Chilonycteris parnellii*) are characterized by

three distinct components — a beginning FM sweep, a long pure tone, and a terminal FM sweep. A bat's own cry evokes two consistent fast wave (N_4) potentials; the first is a response to the beginning of the FM sweep, and the second "terminal" N_4 potential is evoked by the off of the pure tone component and/or by some part of the terminal FM sweep. The "terminal" N_4 potentials were the only type consistently recorded in response to echoes. Echoes overlapping the outgoing pulse or a subsequent pulse did not produce distinct responses, but those returning during an interpulse interval produced high amplitude potentials. These results are consistent with the hypothesis that the bats can control the "echolocative depth of field" by regulating the interpulse interval. Echoes appear to be processed much more efficiently than the outgoing pulses, and this can be readily accounted for by the action of the middle ear muscles. In flying bats the echo-evoked potentials were much higher in amplitude than pulse-evoked potentials.

REFERENCES

1. HENSON, O. W., JR.: The Activity and Function of the Middle Ear Muscles in Echo-Locating Bats. J. Physiol. (London), vol. 180, 1965, pp. 871–887.
2. HENSON, O. W., JR.: The Perception and Analysis of Bio-Sonar Signals by Bats. *In*: Les Systèmes Sonars Animaux. Vol. II. R. G. Busnel, ed., Lab. Physiol. Acoust., INRA-CNRZ, Jouy-en-Josas 78, France, 1967, pp. 949–1003.
3. HENSON, O. W., JR.: Some Morphological and Functional Aspects of Certain Structures of the Middle Ear in Bats and Insectivores. Univ. Kans. Sci. Bull., vol. 42, 1961, pp. 151–255.
4. GRINNELL, A. D.: The Neurophysiology of Audition in Bats: Intensity and Frequency Parameters. J. Physiol. (London), vol. 167, 1963, pp. 38–66.
5. FRIEND, J. H.; SUGA, N.; AND SUTHERS, R. A.: Neural Responses in the Inferior Colliculus of Echolocating Bats to Artificial Orientation Sounds and Echoes. J. Cell. Physiol., vol. 67, 1966, pp. 319–332.
6. GRINNELL, A. D.: Mechanisms of Overcoming Interference in Echolocating Animals. *In*: Les Systèmes Sonars Animaux. Vol. I. R. G. Busnel, ed., Lab. Physiol. Acoust., INRA-CNRZ, Jouy-en-Josas 78, France, 1967, pp. 451–481.
7. LAGERQUIST, L. G.: Neurophysiological Correlates of Acoustic Orientation with Special Reference to the "Off" Response. M.D. Thesis, Yale University, 1969.
8. GRINNELL, A. D.: The Neurophysiology of Audition in Bats: Directional Localization and Binaural Interaction. J. Physiol. (London), vol. 167, 1963, pp. 97–113.
9. GRINNELL, A. D.; AND GRINNELL, V. S.: Neural Correlates of Vertical Localization by Echo-Locating Bats. J. Physiol. (London), vol. 181, 1965, pp. 830–851.
10. NEUWEILER, G.: Neurophysiologische Untersuchungen zum Echoortungssystem der Grossen Hufeisennase *Rhinolophus ferrum equinum*. Z. vergl. Physiologie, Bd. 67, 1970, pp. 273–306.
11. WEVER, E. G.; AND VERNON, J. A.: The Protective Mechanisms of the Bat's Ear. Ann. Otol., Rhinol., and Laryngol., vol. 70, 1961, pp. 1–13.
12. GALAMBOS, R.: Suppression of Auditory Nerve Activity by Stimulation of Efferent Fibers to Cochlea. J. Neurophysiol., vol. 19, 1956, pp. 424–437.