

HEARING IN PRIMITIVE MAMMALS, I:
OPOSSUM (*Didelphis virginianus*)¹

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INTRODUCTION

This report is the first of a series concerned with the general question of the evolution of human hearing. Although the chief goal of the series is to determine if, and along what dimensions hearing capacities have changed during the phylogenetic history of mammals, this line of inquiry might also serve to further comparative audiology by providing a basis for distinguishing generalized or historically persistent auditory characteristics from those that are specialized or sensitive to habitat.

The selection of the common North American opossum as the subject of the first experiment in a series on primitive mammals relies on evidence from morphology, paleontology and paleoneurology. Anatomical comparison has shown that the structure of the opossum ear and brain closely approximates that form which serves as a parsimonious starting point for tracing the evolution of modern mammals (Watson, 1953; Tumarkin, 1955; Smith, 1910; Loo, 1930; Clark, 1934; Olson, 1959; Simpson, 1949 and 1959). Paleontological evidence has demonstrated that the opossum has survived in relatively unaltered form since the Cretaceous, at least 75 million years ago (and probably long before that) (Gregory, 1929). Finally, paleoneurological evidence has revealed that the brain of the primitive placental mammals of the Eocene was strikingly similar to that of the modern (and also the ancient) opossum (Edinger, 1948). Along with the paucity of alternative subjects, these facts indicate that the opossum is, at least neurologically, the most primitive mammal that is available for behavioral testing.

Our ability to measure the auditory capacity of opossums depends on a recent advance in behavioral technique that allows sensory assessment in even the most intractable mammals (Sidman, et al., 1966; Hendricks, 1966).

METHOD

Subjects.

Two adult wild-born and experimentally naive opossums (*Didelphis marsupialis virginianus*) were used, each weighing 3.5-4 kg.

Apparatus.

Behavioral equipment. The animals were trained and tested in a 46cm x 25cm x 46cm chamber located next to a one-way window in a sound-treated room. The ceiling and three walls of the chamber were made of opaque plexiglas. The fourth wall was made of clear plexiglas to permit observation of the animal. The floor consisted of 3/16" brass

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bars on 1/2" centers wired to allow delivery of a shock to the animal's feet. A 1" thick layer of wood shavings lay beneath the gridded floor. A water bottle and pellet dispenser were fastened outside of the chamber to one of the opaque walls. Tubes led from the bottle and the dispenser to a lick-spout and food cup, respectively, which were fastened to the inside of this same wall.

Sound equipment. Sine waves were produced by a Hewlett-Packard 200CD oscillator and led first to a Grason-Stadler 829 E. electronic switch, then to a Hewlett-Packard 350D attenuator and finally, to a University 312 speaker mounted in the ceiling of the test chamber. This system proved capable of delivering undistorted tones over a frequency range from 50 c/s to over 80 kc/s.

Throughout training and testing the electrical signal to the speaker was monitored with an oscilloscope which had previously been calibrated with a sound pressure level (SPL) meter.

To measure true sound pressure and calibrate the sound system and oscilloscope, a Bruel & Kjaer Model 2604 microphone amplifier, with either a 1/4" or 1/2" condenser microphone (Models 4133 and 4136) were used. During calibration, the microphone was placed at several orientations in the same position that was occupied by the animal's head while it was licking. Although standing waves could be detected at various places within the chamber, the area near the animal's head proved to be free of nodes at the frequencies included in the experiments. Throughout this report 0.0002 dynes/cm² is used as an intensity reference level.

Reward and Punishment.

Each reward consisted of a standard 45-mg. food pellet (Noyes). Equal portions of glucose, sucrose, rat and dog pellets were mixed in the hopper of the pellet feeder. This diet was occasionally supplemented by vitamins and a few ounces of horsemeat. Water was always available in the home cages as well as in the test chamber where it served as part of the manipulandum.

During the first few training sessions, a constant-voltage shock seemed to yield more stable suppression than a constant-current shock. Thereafter, a 60-cycle, 2-volt sine-wave of 0.3 sec duration was used as punishment.

Training Procedure.

Psychophysical tests in animals using the technique of conditioned suppression have been described in detail elsewhere (Sidman, et al., 1966; Hendricks, 1966). Briefly, this technique requires the establishment of two responses: an instrumental response and a conditioned emotional response that is incompatible with the instrumental response. In our particular variety of this technique, the instrumental response was the licking of a water spout. This response was trained and maintained by a *food* reward. After an initial shaping procedure, the probability of reward was systematically decreased to 1-2% (VR) of the number of licks.

The second response resulted from pairing a steady pure tone with an unavoidable shock to the feet. As the tone acquired the properties of a warning signal due to its repetitive pairing with shock, the animal stopped licking whenever the tone occurred. During threshold testing, this stoppage or suppression of licking was used as evidence that a tone had been perceived.

Once the animal began to demonstrate conditioned suppression, i.e., stopped licking whenever the warning tone was sounded, the number of trials was fixed at 30 per day with an intertrial interval varied randomly from 30 to 180 sec. It should be

emphasized that throughout training and testing, every presentation of a tone was followed by an aversive shock whether or not the tone eventually proved to be suprathreshold.

Threshold Testing Procedure.

At each frequency, threshold testing was conducted in two ways: first, the threshold was estimated by a modified method of limits, then a second exhaustive determination was made by the method of constant stimuli.

In the first stage of threshold testing, the intensity of the tone was gradually decreased in 5-db steps on each successive trial until a failure to suppress was observed. The intensity of the tone was then increased in 5-db steps on each successive trial until obvious suppression once more occurred. By repeating this procedure a number of times the threshold could be readily estimated.

In the second stage, tones with intensity levels at 5-db increments extending from at least 10 db below to 10 db above the estimated threshold were presented in random order. After at least 5 and usually 10 presentations at each of these preselected levels, a smooth psychophysical curve of suppression as a function of intensity usually could be plotted. This entire procedure was then repeated until successive spot-checks and replications revealed no apparent shift from the previously determined threshold values.

Finally, for reasons given below, we felt it advisable to demonstrate that a frequency limen existed at high frequencies. In this special procedure a method of limits was used once more. The animal was presented with 1/sec tone pips each 0.7 sec in duration. After the animal extinguished its previously conditioned alarm to this now neutral stimulus, shock was paired with 10-sec train of tone pips which alternated between the test stimulus ($F + \Delta F$) and the neutral stimulus (F), high-low-high-low . . . , without interrupting the 1/sec rhythm. When this discrimination produced reliable suppression, the value of ΔF was systematically varied over successive trials in the same manner as intensity had been varied in the previous testing of absolute intensity limens.

Analysis of Data.

The use of the technique of conditioned suppression for the assessment of sensory thresholds hinges on interpreting a change in the rate of the instrumental response—in this case the rate of licking—as evidence that the subject has detected the stimulus. The many practical and theoretical questions surrounding the use of this technique have been treated at length elsewhere (Sidman, et al., 1966; Hendricks, 1966). For this report, we have chosen a unitless measure of performance which compares the number of licks in the 10-sec warning period in which the stimulus was being presented (designated W) to the number of licks in the 10-sec safe period immediately preceding the warning stimulus (designated S) according to the formula:

$$\text{Discriminatory performance} = 1 - (W/S) = (S-W)/S$$

Although this measure is bounded above by +1.0 and unbounded below, for a trained animal it varies only from about -0.2 to +1.0. A value of +1.0 indicates perfect suppression and is interpreted to mean that the stimulus was heard by the subject whenever it was presented. A value near zero indicates that the rate of licking did not change significantly upon presentation of the stimulus and is interpreted to mean that the stimulus was not heard by the subject. Performance values between 0 and +1.0 are interpreted to mean that the subject may have heard, or only occasionally heard, the

stimulus. For the construction of audiograms, we have arbitrarily chosen a performance value of + 0.5 as threshold.

RESULTS

Figure 1 shows the psychophysical function generated by one opossum at 60 kc/s. The figure also illustrates the method used in selecting a threshold for the construction of audiograms.

Figure 2 shows the audiograms of the two opossums. The two curves agree quite closely, deviating noticeably from each other only at the highest frequencies tested.

Perhaps the most striking feature of the results illustrated in Fig. 2 is the ability of the opossums to hear high frequencies. Although from previous observations we suspected that the opossum could hear frequencies beyond the range of human hearing, we were completely unprepared for the discovery that their upper limit might be two octaves above ours. Therefore, we entertained the possibility that the opossum's sensitivity to very high frequencies might be due to some sort of pseudo-hearing, such as an ability to detect the envelope of high frequency sound waves rather than the sound waves themselves (Cf. Roeder and Treat, 1961; Corso, 1963). This possibility led us to include a measurement of ΔF at a high frequency. We reasoned that if the opossum was merely detecting the envelope of pressure changes or some other parameter that is largely independent of frequency, then a ΔF at high frequencies would be either very large or non-existent.

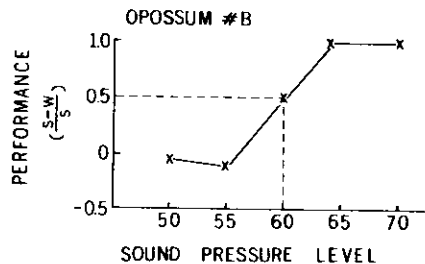


Fig. 1. Lick suppression at 60 kc/s as a psychophysical function of sound pressure level. Dashed line indicates method of choosing a sound pressure level for construction of audiogram.

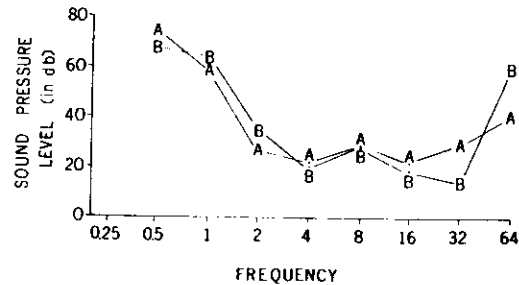


Fig. 2. Audiograms of opossums from 0.25-64 kc/s. Note high-frequency sensitivity but low sensitivity overall.

Figure 3 shows the results of the ΔF test in kc/s at a frequency of 42 kc/s and an intensity 40 db above absolute threshold. The results clearly show that a ΔF at high frequencies does exist and is reasonably small. This result leads us to conclude that the opossum probably does not depend on an unusual mechanism for high-frequency hearing.

DISCUSSION

The results show that the opossum hears pure tones ranging in frequency from .5 to 60 kc/s. If the audiograms are extrapolated to 80 db, the frequency range of audibility can be conservatively estimated to extend from about .250 to 70 or 80 kc/s.

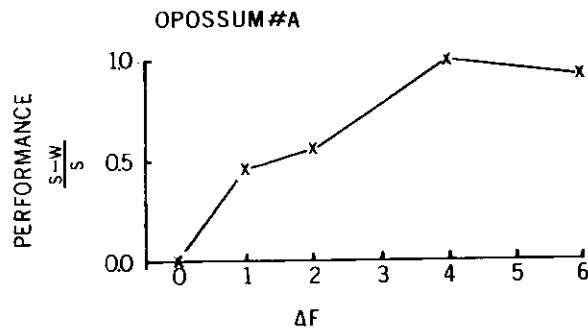


Fig 3. Lick suppression at 42 kc/s as a psychophysical function of frequency increments in kc/s.

Two features of the audiogram merit special comment. First, the opossum hears tones of very high frequency. Second, the opossum is relatively insensitive to pure tones throughout its frequency range.

High Frequency Hearing.

Although the two audiograms show a 14 db disparity at 32 kc/s and a 20 db disparity at 60 kc/s, there is little question that both opossums could hear these tones. In addition to the fact that the psychophysical functions of suppression ratio and intensity followed the same form as they did at lower frequencies (Fig. 1), direct observation of the subjects during high frequency testing revealed the same stereotyped alarm reaction as had been observed whenever a clearly suprathreshold tone was presented. For these reasons we feel that the high frequency points on the audiograms warrant the same degree of confidence as each of the low frequency points.

The further question of whether or not the mechanism of hearing at high frequencies is similar to that at low frequencies is not answered by these experiments. However, the possibility that the opossum merely responds to the envelope of pressure waves instead of the pressure waves themselves is contraindicated by the existence of a reasonably narrow ΔF . Thus, we have no reason to believe that the opossum's high frequency mechanism may be different from its low frequency mechanism.

Finally, it should be noted that the disparity between the two audiograms increases at high frequencies. This result is not uncommon—it usually proves to be the major source of variance between audiograms of individuals in any species, including man. For this reason, we interpret the increase in disparity at 32 and 60 kc/s as further evidence that the opossum's upper limit of hearing is nearby. Thus, in spite of its sensitivity at these frequencies, the opossum's upper limit falls far short of mammals, such as bats or dolphins, that have evolved mechanisms for echo-location (Dalland, 1965; Schevill and Lawrence, 1953).

Overall Sensitivity.

The vertical or intensity coordinates of the lowest points in the audiograms in Figure 2 range from 15 db to 30 db above $0.0002 \text{ dynes/cm}^2$. Even if a less conservative definition of threshold is used, e.g. a performance value of 0.2 instead of 0.5, these values remain sufficiently high to warrant the conclusion that opossums are insensitive compared to most other mammals (Cf. Gourevitch and Hack, 1966; Neff and Hind, 1955; Sivian and White, 1933; Fujita and Elliot, 1965; Stebbins, et al., 1966).

This relative insensitivity of the opossum is directly supported by electrophysiological recordings of the cochlear microphonic potential (Fernandez and Schmidt, 1963; McCrady et al., 1937 and 1940). It is also, though indirectly, supported by anatomical differences between the structure of the middle ear of opossums and other mammals. Unlike the ear of advanced mammals, the tympanic membrane of the opossum is attached at its circumference to a thin ring which, in turn, has no bony support of its own (Watson, 1953; Tumarkin, 1955). Thus, it is not unlikely that the loss of energy at the tympanum in opossum is greater than it is in mammals with tympanic rings that are more firmly attached.

The thin tympanic ring of opossum is just one of the primitive structural features which have led comparative anatomists to consider the opossum ear as an approximation to a form which was historically intermediate to reptiles and advanced mammals (Le Gros Clark, 1934). In this regard it is particularly satisfying to note that the overall sensitivity of the opossum, even though it is poorer than that of other mammals, is better than any non-mammal that has yet been tested (McGill, 1960; Schwartzkopff, 1955).

SUMMARY

Using the technique of conditioned suppression, two wild-born opossums were trained to respond to the presence of pure tones. Performance as a psychophysical function of intensity was then plotted at 8 frequencies. The results show that opossum is sensitive to frequencies from 0.5 to 60 kc/s and probably hears frequencies from .25 to 70 kc/s at 80 db sound pressure level. The opossum is relatively insensitive compared with other mammals throughout most of its range of audibility.

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