was used with mice treated with puromycin immediately (4 to 8 minutes) after training; they were trained in a Y-maze to a criterion of nine correct responses out of ten, shock being given for failure to move from the stem of the Y within 5 seconds and for errors in left-right discrimination. The same procedure was used in tests for retention of the training experience. The retention tests were given 5 days after treatment with saline; in control groups without saline, they were given at corresponding times.

In the presence of puromycin our mice, when naive to the maze, are exceptionally difficult to train. This difficulty was avoided by training in two stages: The mice were first trained to avoid to criterion in the maze, with free choice of either arm of the Y. Next day they were injected with puromycin and 5 hours later, when inhibition of protein synthesis was at its height (*3*), they were trained not to enter the preferred arm in the previous free-choice situation. During discrimination-training in this group, errors of avoidance were infrequent and, because of the plan of the experiments, percentages of savings in the retention tests were calculated on the basis of errors of discrimination. An insignificant difference in the results was introduced by the basing of savings on errors of both avoidance and discrimination.

Table 1 gives the results. Of the 14 control mice that received puromycin after avoidance-training and before discrimination-training (group A), but no saline subsequently, all but one failed to show memory of training when tested for retention. By contrast, eight of 17 mice treated with saline showed varying degrees of retention. Statistical analysis (*t*-test), however, gave no convincing evidence that in savings the saline-treated mice differed significantly from their controls: the percentages of savings of trials and errors for the controls were, respectively, 8.5 ± 5.6 and 7.1 ± 7.0 percent (means ± S.E.); the corresponding figures for mice treated with saline were 27.8 ± 10.0 and 35.2 ± 10.3 percent. The difference in savings of trials between the two sets of mice was not significant ($P > .1$), while the difference in savings of errors was marginally significant ($P < .05$).

The mice of group B (Table 1) were injected with puromycin immediately after training. In this instance, all control mice (no saline) when tested for retention had lost memory of training, while 15 of 19 mice treated with saline showed varying degrees of retention. In the controls, the percentages of savings of trials and errors were, respectively, 1.2 ± 1.5 and 3.2 ± 2.3 percent (means ± S.E.); in the mice treated with saline the corresponding figures were 43.8 ± 8.9 and 54.6 ± 7.9 percent. The difference between the two groups in savings of trials and errors was clearly significant ($P < .01$).

Our earlier results on the efficacy of saline in restoring memory after treatment with puromycin referred to 47 mice in which puromycin was given 1 or more days after training and was followed 30 hours to 60 days later by intracerebral injections of saline. In these mice the percentages of savings of trials and errors were, respectively, 66.2 ± 4.6 and 76.8 ± 3.2 percent (means ± S.E.). These savings are marginally significantly greater ($P < .05$) than those obtained when puromycin, given immediately after training, was followed 5 days later by saline.

We conclude that treatment with puromycin either before or immediately after training interferes with the process responsible for establishing the basic memory trace. This effect is apparently stronger when puromycin is given before training, although, because of differences in the training procedures, comparison of the two groups is uncertain. One should note that, although puromycin was given immediately after training, this fact does not mean that interference with consolidation of memory started at this time; the mechanism of action of puromycin on memory must be better understood before the time available for consolidation can be estimated in such a situation.

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## Auditory Sense in Certain Sphingid Moths

Abstract. *Moths of the genus* Celerio, *hovering and feeding from blossoms, have been observed to react vigorously to high-pitched sounds. The acoustic receptor appears to involve the labial palps. Its characteristics have been determined from responses made, when the moth is exposed to artificial sound pulses, by an interneuron originating in its head. These responses have been found only in 4 out of 19 sphingid species tested.*

Moths belonging to the family Sphingidae lack the thoracic or abdominal tympanic organs found in several other families (*1*). In Noctuidae there is evidence that these tympanic organs serve as receptors for the ultrasonic cries of insectivorous bats (*2*) and that they mediate various kinds of evasive behavior (*3*). These findings, together with the lack of any recent observations (but see *4*) that sphingid moths react to high-pitched sounds, have given rise to the belief that sphingids lack auditory organs. Indeed, such a warning system might seem superfluous in those sphingid species that are large and powerful fliers and hence unlikely to fall prey to insectivorous bats.

In March 1967, one of us (K.D.R.) had the opportunity to watch about a dozen individuals of *Celerio lineata* Fabr. hovering, as they fed on nectar, in front of a trellis covered with jasmine. A slight "hiss" or a jingle of keys caused the moths to dash away at high speed, only to return and resume feeding in 10 to 15 seconds. This observation has been repeated with *C. euphorbiae* Linn. in captivity. The moths, confined in a cage, could be induced to hover and feed on the nectar in phlox blossoms. A high-pitched sound would abruptly terminate this feeding behavior and cause wild dashing flight lasting several seconds. When at rest, or when restrained for closer observation, the moths gave no behavioral response to ultrasound.

Neurophysiolgical studies carried out on these two species of *Celerio* show that there is a descending spike response in an axon in the cervical connectives when ultrasonic pulses from a loudspeaker are directed at the moth (Fig. 1). The latency of this spike response (6 to 8 msec) and other characteristics suggest that it belongs

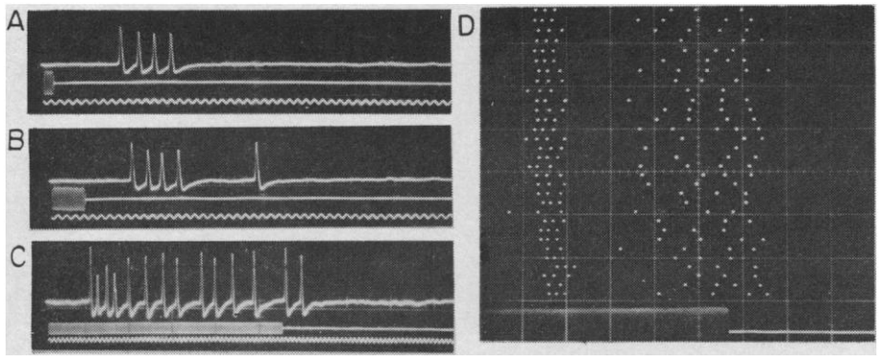


Fig. 1. Responses of a descending interneuron to ultrasonic pulses. Spikes were recorded from an insulated metal microelectrode inserted in the neuropile of the prothoracic ganglion. (A) *Celerio lineata*; response to a single pulse of 40 khz, 1 msec in duration, and +60 db in intensity (middle trace). Time marker, 1 khz (lower trace). (B) The same, duration of stimulus pulse 4 msec. (C) The same (on contracted time base), response to stimulus pulse 55 msec long. (D) *C. euphorbiae*; each line of dots marks the spike responses to one each of a series of pulses of 60 khz, +60 db intensity, and 55 msec duration (bottom trace) repeated at two pulses per second. Vertical grid lines mark 10-msec intervals.

to a stable second-order neuron similar to the noctuid repeater neuron (*5*) but having its origin in the head of the moth.

The following properties of the acoustic receptor have been deduced from the spike response of this interneuron. The minimum sound intensity producing a stable response in the interneuron is +50 db (relative to 0.0002 dyne/cm$^2$). This value indicates that the sphingid receptor is 10 to 15 db less sensitive than the most sensitive noctuid tympanic organs (*6*). However, in assessing this figure one must remember that the measurements on noctuids were made with the first-order spike response in the acoustic nerve fibers used as criterion. Maximum sensitivity of the sphingid auditory organ is reached at frequencies between 20 and 40 khz. However, responses were obtained to sound pulses ranging from 7 khz to over 100 khz. The sensitivity to lower frequencies seems to be greater than that found in noctuids (*6*); the chirping of a sparrow outside the laboratory was an adequate stimulus in one instance. Pulsed tones shorter than 1 msec may elicit a train of three or four spikes in the interneuron (Fig. 1, A and B). However, the response is not merely to transients in the stimulus; longer tones (55 msec) elicit a succession of spikes in the interneuron (Fig. 1C), sometimes with a pause in the middle of the train (Fig. 1D).

The following experiments indicate that the interneuron response is initiated when acoustic energy impinges on the labial palps. Amputation of the antennae or tongue has no effect on the response. Removal of one palp and amputation of the other in short segments beginning at the tip causes losses in acoustic sensitivity proportional to the amount amputated. When only a short stump of the palp remains, acoustic sensitivity has dropped 100-fold or more, although other characteristics of the response appear to be unchanged. The receptor mechanism appears to be located near the point where the palp articulates with the cranial skeleton. Repeated attempts failed to detect acoustically elicited spike responses in the palp nerve as it runs through the basal segment. Such a response might have been expected if the receptor mechanism is located on the second or third segments of the palp. However, deflection of the intact palp a few degrees to the right or left abolishes the response of the interneuron to ultrasound. Previous sensitivity returns as soon as the mechanical impediment to palp vibration is removed.

The form of the labial palp in sphingid species showing the acoustic response described above was compared with that in sphingid species that lack such a response. The second palpal segment in the Choerocampinae is large and bulbous (Fig. 2A) and lacks scales on its thin, flattened inner surface. Dissection and histological sections show that the first and second segments consist largely of an air sac (Fig. 2B) with a narrow blood space and nerve running along the dorsal surface. Thus, the palp must be relatively buoyant, and the lack of scales on its inner surface must reduce damping friction with the tongue. This makes it seem possible that the palps are displaced by acoustic energy and that this displacement is sensed by a mechanoreceptor located near the point where the palp articulates with the cranium.

The distribution of this auditory sense among subfamilies of the Sphingidae was surveyed with the response of the interneuron in the cervical connectives used as criterion. Nineteen species were examined, some only as single individuals. In the Choero-

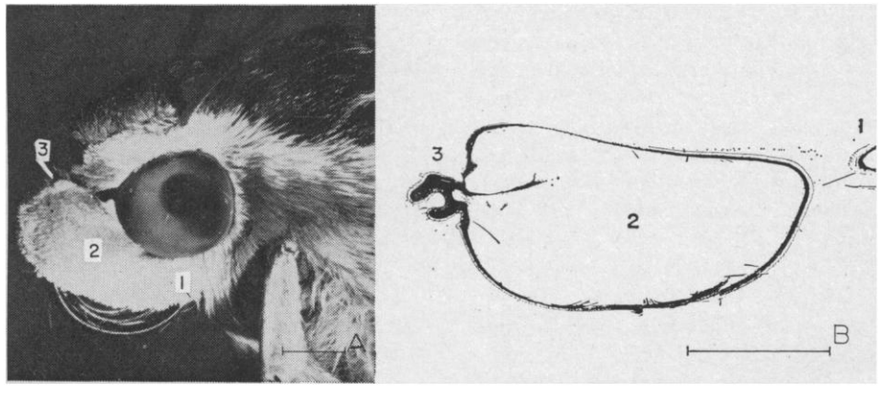


Fig. 2. (A) Lateral view of the head of *Celerio lineata*. The labial palps (the left is visible covered with scales) extend forward from their articulations below and medial to the posterior margins of the compound eyes. They lie on either side of the coiled tongue. The greater portion of the palp is made up of the enlarged second segment (2). The small third segment (3) is just visible on the anterior dorsal part of the second segment. The articulation of the first segment (1) with the second is hidden by scales. (B) View (low-power) of a longitudinal section through the left labial palp. Segments numbered as above. The section misses the articulation of the second with the first segment, of which only a small part is visible at the right. The greater part of the second segment is occupied by a tracheal air sac. External cuticle, transparent layer bounded by thin black line; tracheal epithelium and other tissue appears black. Scales on A and B, 1 mm.

campinae all the available species, *Celerio lineata* Fabr., *C. euphorbiae* Linn., and *Xylophanes tersa* Linn., showed positive responses. Acoustic responses were lacking in Sphinginae (six species), Smerinthinae (three species), and Macroglossinae (two day-flying species). Out of five species of Philampelinae tested only *Erinnyis obscura* Fabr. showed a response which required, however, sound intensities higher than those eliciting responses in choerocampine moths.

The functional significance of this species distribution is not clear. The habit of feeding on nectar while hovering with extended tongue is common to many diurnal and crepuscular sphingids, and might be expected to expose these otherwise swift fliers to attacks from birds or bats. Yet no obvious correlation could be found between this habit and tongue length and the possession of an auditory sense. Body size offers no answer; the choerocampine sphingids examined are intermediate in body size, many other sphingids being larger and many smaller. Comparative studies of the biology and behavior of adult sphingid moths may throw some light on the matter.

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## Ranger VIII and Gravity Scaling of Lunar Craters

Most investigators agree with Baldwin (*1*) on the location of the crater produced by the impact of Ranger VIII, but several points regarding the predicted size of the crater and its morphology need clarification. Baldwin suggests (i) that the size of the crater can be compared with craters produced by explosives detonated at scaled depths ($H/W^{\frac{1}{3}}$; $H$ being the depth in feet, and $W$ being the weight in pounds of the TNT equivalent) of burial between 0.10 and 0.25 ft/lb$^{\frac{1}{3}}$, and (ii) that the diameter of the crater should be 1.569 times larger than its terrestrial counterpart. It is doubtful that either of these suggestions is correct because Ranger VIII hit the lunar surface with an oblique trajectory, and data on cratering show corrections for the acceleration of gravity at the lunar surface are less than 1.569. In addition, the choice for the Ranger VIII impact crater shown by Baldwin is strengthened by the fact that it meets two requirements, namely, the distribution of ejecta around the crater is asymmetrical and the crater itself is oval.

The conditions of impact of Ranger VIII are similar to those of missiles at White Sands, New Mexico. In both cases, comparable trajectories are involved. Ranger VIII hit the lunar surface at 2.653 km/sec and an angle of 41.6° from the local horizontal (*2*). Some missiles at White Sands impact alluvium, lake beds, and fixed dunes with velocities near 2.6 km/sec and angles of impact of 42° to 58°. Thus, there are experimental data on impacts with natural materials for comparison with the crater produced by the impact of Ranger VIII.

The sizes of craters produced by missiles with oblique trajectories impacting at sufficiently high velocities are comparable to the sizes of craters produced by chemical explosives with small-scaled depths of burial when the kinetic energy of the missile is the same as the TNT (trinitrotoluene)-equivalent energy release of the explosive (*3*). Uncertainties in the direct correlation between impact craters and explosive craters arise, however, when the impact craters are produced by projectiles with oblique trajectories. Additional uncertainties arise from variations in crater size that are dependent on projectile velocity and target properties. In Fig. 1, diameters of missile impact craters in natural materials, measured from rim crest to rim crest, are compared with the kinetic energies of "inert" missiles impacting at oblique angles. Extrapolation of the data with "cube root" scaling (diameter proportional to the cube root of the kinetic energy) indicates the diameter of a crater produced by the impact of Ranger VIII on Earth should be between 7.0 and 12.5 m across but probably near 9.5 m. If gravity scaling (diameter proportional to the fourth root of kinetic energy) were used, the minimum predicted diameters would be about 6 to 11.5 m.

Since the acceleration of gravity at the lunar surface is about one-sixth of that at the earth's surface, these diameters might differ. The maximum correction for diameters of craters that are hemispherical or of other dimensions with fixed ratios is 1.569. We may compute

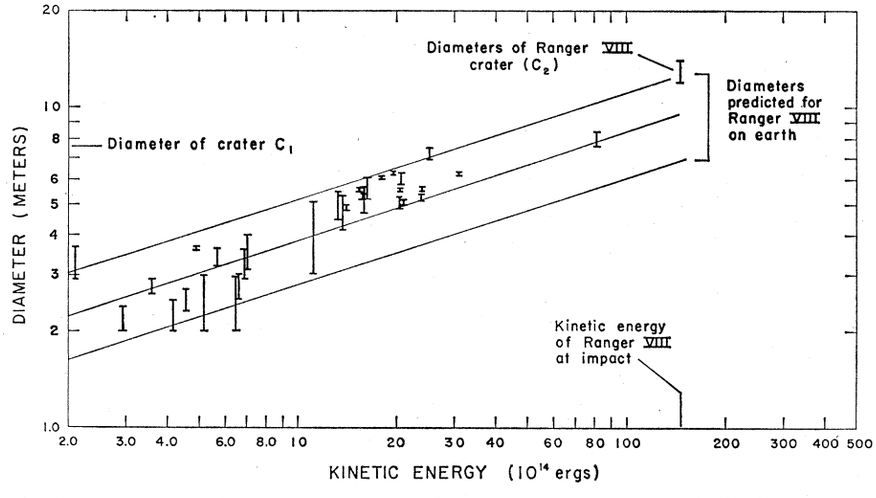


Fig. 1. Comparison between diameters of craters produced by missile impacts and diameter of crater produced by Ranger VIII. Missiles impacted at angles between 42.3° and 58° from the horizontal; Ranger VIII impacted at 41.6°.