as they characterize the tumor's sensitivity to the drug, may be invaluable in the search for added benefits from chronotherapy based upon the chronobiology of the tumor as well as that of the host.

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Neural Attenuation of Responses to Emitted Sounds in Echolocating Bats

Abstract. *Bats of the family Vespertilionidae emit strong ultrasonic pulses for echolocation. If such sounds directly stimulate their ears, the detection of echoes from short distances would be impaired. The responses of lateral lemniscal neurons to emitted sounds were found to be much smaller than those to playback sounds, even when the response of the auditory nerve was the same to both types of sounds. Thus, responses to self-vocalized sounds were attenuated between the cochlear nerve and the inferior colliculus. The mean attenuation was 25 decibels. This neural attenuating mechanism is probably a part of the mechanisms for effective echo detection.*

Bats of the family Vespertilionidae emit short ultrasonic pulses for echolocation. The amplitude of these sounds is about 110 to 120 db SPL (sound pressure level referred to 0.0002 dyne/cm^2 root-mean-square) at 3 to 10 cm from the mouth (*1*). If such strong outgoing sounds directly stimulate the ears, echoes from short distances would be difficult to detect, because the response is very poor to a sound that immediately follows another (*2–4*). Bats have the following mechanisms to reduce the degree of selfstimulation. (i) The inner ear is loosely attached to the skull, so that the amount of bone conduction is minimized (*5*). (ii) The middle ear muscles contract synchronously with vocalization and attenuate the amount of self-stimulation by as much as 15 to 20 db [*Tadarida brasiliensis* (*6*)]. (iii) The sensitivity of the auditory systems of *Rhinolophus ferrum equinum* and *Chilonycteris parnellii parnellii* is low for the sound frequencies emitted during flight, but sensitivity is high for sounds a few kilohertz higher than the emitted sounds, such as an echo shifted by the Doppler effect (*7*). In addition, there may be a neural mechanism by which responses of auditory neurons to outgoing sounds are reduced. We report evidence for the presence of such a neural attenuating mechanism in the brain of bats of the genus *Myotis*, which emit short frequency-modulated (FM) sounds for echolocation.

Nails 1.8 cm long were mounted with dental cement on the skulls of 16 gray bats (*Myotis grisescens*) under ether anesthesia. In the following days, the awake animal was placed on a plastic ball floating on water in a soundproof room. The nail was tightly held by a metal rod mounted on a micromanipulator. Without anesthetic, three small holes were made in the skull, and a pair of sharpened steel electrodes and two tungsten electrodes were inserted. The steel electrodes were used to stimulate vocal areas in the midbrain with a train of short elecrtic pulses delivered 2 to 3 sec^{-1} (*8*). One of the tungsten electrodes was inserted into the auditory nerve (or cochlear nucleus) in order to record N_1, the summated activity of the auditory nerve fibers. The other was inserted into the nucleus of the lateral lemniscus on the contralateral side in order to record LL, the summated activity of the laternal lemniscus (*9*). Thirty-two different sounds emitted by every bat were monitored with a ¼-inch microphone (Brüel & Kjaer 4135) placed 5 to 8 cm in front of the bat's mouth and were recorded with a tape recorder with a frequency response of 50 to 150,000 hertz (Ampex FR-100). The N_1 and LL evoked by these 32 emitted sounds were averaged by a computer (Nicolet 1070) and plotted with an X-Y recorder (Fig. 1A); these are called "the self-evoked N_1 and LL." The same 32 sounds were immediately played back at different amplitudes through a loudspeaker 68 cm in front of the bat's head. The pressure level of these sounds was monitored with the ¼-inch microphone, placed near the bat's ear. The N_1 and LL evoked by these playback signals were also averaged and plotted; these are called "the playback N_1 and LL." The self-evoked and playback LL were compared in order to determine whether their amplitudes were different when N_1 was the same for the emitted and playback sounds. Since the computer was synchronized to the onset of either the emitted or the playback sounds, fluctuations in time were always present

in averaging the responses, but its amount was the same for both the self-evoked and playback activities. The standard deviation of the amplitudes of these evoked potentials was about ± 5 percent.

The playback sounds were similar to the emitted sounds but not identical because of the characteristics of the loudspeaker and noise in the recording system. The loudspeaker had a frequency-response curve flat within ± 2 db between 22 and 100 khz. The playback sounds were always accompanied by continuous noise originating in the recording system, so that the signal-to-noise ratio was 30 to 40 db, much smaller than that for the emitted sound (greater than 60 db). The continuous noise might interfere with the responses. In order to deliver sounds similar to orientation sounds and with a large signal-to-noise ratio, 32 FM signals were produced with electronic instruments, and N_1 and LL for them were averaged and plotted; these are called "the artificial N_1 and LL." In these artificial sounds, the signal-to-noise ratio for a signal of 100 db SPL was about 80 db, the duration was 2 msec, and the rise-decay time was 0.2 msec.

When the dorsolateral part of the reticular formation below the boundary between the superior and inferior colliculi was electrically stimulated, the bat emitted a short FM sound of 105 to 115 db SPL with a latency of 40 to 60 msec (*8*). The FM sounds evoked by successive stimuli showed nearly identical properties in overtones and envelope, although the amplitude and duration of successive sounds varied to some extent. In each FM sound, frequency changed from about 100 to 40 khz. Figure 1A shows N_1 and LL evoked by such emitted sounds (a) and playback sounds at different pressure levels (b and c). The amplitude of playback N_1 is nearly the same as that of the self-evoked one. Playback LL is, however, much larger than that of the self-evoked one (*t*-test for amplitude difference, $P < .01$). This indicates that although primary auditory neurons are excited to nearly the same extent by both the emitted and playback sounds, the activity in the lateral lemniscus is different depending on whether the animal vocalizes or the sound is delivered from an external source. The bat has thus some neural mechanism for the attenuation of the amount of excitation of auditory neurons between the auditory nerve and the inferior colliculus.

In order to know the amount of attenuation in decibels, the pressure level of the playback sounds was systematically changed and the amplitudes of N_1 and LL were measured. In Fig. 1B, the two curves show the relation between the approximate pressure level of the playback sounds and the amplitudes of playback N_1 and LL. The dashed line indicating the amplitude of the self-evoked N_1 crosses the curve for playback N_1 at 71 and 103 db SPL. The dashed line indicating the amplitude of the self-evoked LL crosses the curve for playback LL at 51 db SPL. If the self-evoked N_1 was elicited by the emitted sounds of either 71 or 103 db SPL at the input of the inner ear, the self-evoked LL would indicate that the amount of neural attenuation is

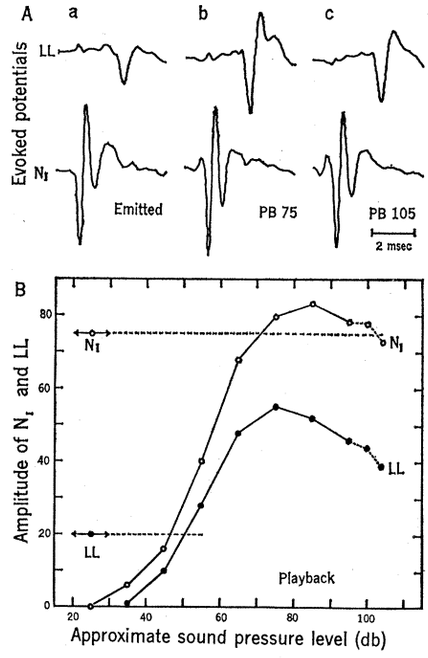

Fig. 1. (A) Summated activities of the auditory nerve (N_1) and lateral lemniscus (LL). Potentials were evoked by sounds emitted by the bat (a) and by playback (*PB*) sounds from a tape recorder (b and c). The amplitudes of the playback sounds were approximately 75 db SPL for (b) and 105 db SPL for (c). The self-evoked LL in (a) is much smaller than the playback LL in (b) and (c), although the self-evoked N_1 in (a) is nearly the same as the playback N_1 in (b) and (c). (SPL: sound pressure level referred to 0.0002 dyne/cm² root mean square. (B) The relation between the amplitudes of the playback N^1 and LL and the approximate pressure level of the playback sounds. Potentials are in arbitrary units. The amplitudes of the self-evoked N_1 and LL are indicated by the horizontal arrows and dashed lines. The amount of neural attenuation is 20 db. (The animal was not anesthetized or immobilized, but awoke during the measurements.)

either 20 or 52 db. According to Henson (*6*), the SPL of emitted sounds at the input of the inner ear is about 30 db weaker than that monitored with a microphone placed 5 to 10 cm in front of a bat's mouth; this is caused by contraction of the middle ear muscles which is synchronized with vocalization and by the tissue isolating the ear from the vocal tract. Since the emitted sounds in our experiments were 105 to 115 db SPL at 5 to 8 cm from the bat's mouth, the amount of self-stimulation was probably 75 to 85 db SPL at the input of the inner ear. The amount of the neutral attenuation was then 20 db.

When artificial FM signals similar to sounds emitted by the bat were delivered, the LL evoked by them was much larger than the self-evoked LL even when the amplitude of N_1 was the same for the emitted and artificial sounds. The amount of neural attenuation was about 30 db. Thus, comparison of the self-evoked N_1 and LL with the artificial ones also indicated the presence of some neural attenuating mechanism operating simultaneously with vocalization.

Because vocalization was initiated by the electric stimuli applied to the midbrain, there was a possibility that the neural attenuation was associated with the electric stimuli (*10*), not necessarily with vocalization. When the ball floating on water was moved or was removed from beneath the bat, the animal emitted several FM sounds. When poked with a forceps, the animal squeaked. Comparison of the self-evoked N_1 and LL in those conditions with the playback ones also showed the presence of neural attentuation, so that it was associated with vocalization regardless of the type of emitted sound. The amount of neural attenuation in 41 experiments with 16 awake bats ranged between zero and 40 db. Its mean value was 25 db.

How is the neural attenuation produced? There are at least two possible mechanisms. In one of them, a vocal center (or centers) sends impulses to the auditory nuclei in order to suppress their activities. In the other, the vocal center activates the descending auditory system, which in turn suppresses the activity in the ascending auditory system. The mechanisms for neural attenuation are not yet known, but it is clear that the suppression occurs between the cochlear nerve and the inferior colliculus.

The neural attenuation found in our experiments is probably mediated by

inhibitory neurons. If a strong inhibitory process synchronized with vocalization lasts for a long time, echo detection may not necessarily be improved, so that inhibition should have a short duration. We often noticed that the large LL was evoked by an echo coming back about 4 msec after vocalization, despite the small N_1 evoked by this echo. We guess that the duration of the inhibitory period is very short.

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9. Since the fourth positive evoked potential recorded with an electrode placed on the dorsal surface of the inferior colliculus was named N_4 [A. D. Grinnell, *J. Physiol. London* **167**, 38 (1963)], it has been erroneously called the collicular evoked potential, although it mainly orginates from the lateral lemniscus (*4*). Thus, if we call N_4 the evoked potential of the lateral lemniscus, it may cause confusion. In our experiments, the evoked potential of the lateral lemniscus was recorded with an electrode inserted into its nucleus, so that its waveform and polarity were not necessarily the same as those of N_4. Because of these two reasons, the potential we measured is called LL rather than N_4.
10. Electric stimuli to certain parts of a brain reduce ascending sensory signals [D. J. Mayer, T. L. Wolfle, H. Akil, B. Carder, J. C. Liebeskind, *Science* **174**, 1351 (1971)].
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Goldfish Retina: Sign of the Rod Input in Opponent Color Ganglion Cells

Abstract. *After light adaptation, all "on-center" ganglion cells in the dark became "red on-center," and all "off-center" cells turned into "red off-center" cells. On a chance basis, this similitude of effect between the rods and the red cones in opponent color cells was not expected. These findings indicate that in the goldfish there is some similarity between the connections of the rods and of the long-wavelength cones.*

With respect to their receptor input, ganglion cells found in the retina are of three types: one type receiving a pure cone input, a second type receiving a pure rod input, and a third type receiving a mixed rod and cone input. In duplex retinas, the second type is generally absent and the third type is the most common. If the cones contain pigments having different spectral absorption characteristics, ganglion cells usually show an opponent color behavior (*1*).

In certain ganglion cells a color opponent mechanism is found to be present, both in the center and in the periphery of the receptive field of the ganglion cells. Such cells are common in the goldfish retina (*2*). An interesting question arises regarding the type of connection that the rod input has on such cells. For example, in a ganglion cell showing in the center of its receptive field a red-green opponent mechanism, where the red light produces an ON response and the green light an OFF response, there exist two distinct possibilities: the rod input can, in the center of the field, produce either an ON response as do the red cones or an OFF response as do the green cones. Of course, the same possibilities exist in the surround. Neglecting the blue cones, one could predict for such a cell equal probability for the rod input to be excitatory like the red cones, or inhibitory like the green cones.

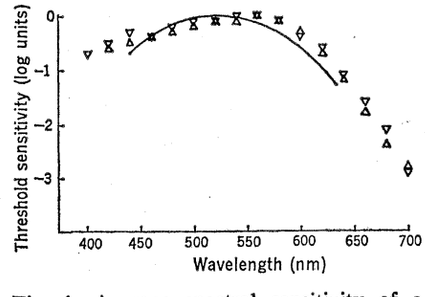

Fig. 1. Average spectral sensitivity of a few dark-adapted cells. Triangles pointing up represent on-center cells, and triangles pointing down, off-center cells. The solid line was generated from a nomogram for a pigment with its maximum at 522 nm. The shift of the experimental curve toward the long wavelengths could be attributed to a contamination from the red cone input.

To provide an answer to this question, a series of experiments was undertaken on the isolated retina (*3*) of the Comet goldfish (*Carassius auratus*). Single-unit activity from ganglion cells was recorded extracellularly with tungsten microelectrodes. In order to maximize the probability of a complete characterization, both in the dark-adapted state and after light adaptation, I chose only cells having a spike amplitude greater than 100 μv for study. Since regeneration of the rod pigment is very limited in an isolated retina, I had to study the dark-adapted characteristics first and then proceed to the light-adapted retina. This limited the study to one cell per retina.

Recordings from 20 cells (12 on-center, 8 off-center), which lasted more than 1 hour each, provide the following picture. All cells have a center-surround organization in the dark-adapted state, with the center either ON or OFF to all wavelengths and the surround with an antagonistic effect to the center. The evidence for a rod input in the dark-adapted state is from (i) the low threshold required to elicit a response (less than 10^6 quanta cm^{-2} sec^{-1}, (ii) the required light adaptation period before the opponent color responses can be observed at an intensity 3 log units above the rod threshold, and (iii) the spectral sensitivity measurements, as shown in Fig. 1. The shift of the experimental points toward the red is probably due to a red cone contamination because of the high cone/rod ratio (7/32) in *Carassius* (*4*). In terms of the center response only, all cells that are ON in the dark-adapted state became ON to red and OFF to green after light adaptation. Similarly, all OFF cells became OFF to red and ON to green. In the light-adapted state, these cells are all of the type described by Daw (*2*), having a color opponent organization both in the center and in the surround and a neutral point around 560 nm, indicating a red-green opponent cell. Figure 2 shows the complete response of these cells when one adds the rod input.

Because of the spectral absorption