in the cerebro-hepato-renal syndrome is not known.

A direct link between peroxisomes and mitochondria exists in plants (*18*), yeast (*19*), and protozoa (*17*). In these cells peroxisomes synthesize succinate which is further metabolized by mitochondria. This pathway has not been demonstrated in higher organisms, and the relation, if any, between the defects in these organelles in the cerebro-hepato-renal syndrome is not clear. The mitochondrial defect could represent lack of an element, presence of an abnormal constituent, or the effect of an inhibitor (*11*).

Volpe and Adams (*3*) have suggested that a neuronal migration defect is the fundamental one in the cerebro-hepato-renal syndrome. Our studies, demonstrating organelle pathology in brain, liver, kidney, and muscle point to an underlying subcellular defect in this inherited disorder.

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Velocity and Displacement Responses in Auditory-Nerve Fibers

Abstract. *With the help of nonsinusoidal acoustic stimuli, it is demonstrated that most fibers of the auditory nerve respond to both displacement and velocity of the basilar membrane. Except at very high stimulus levels, motion and displacement toward scala tympani produce excitation; motion and displacement toward scala vestibuli produce inhibition. The displacement and velocity responses interact. When both are excitatory or inhibitory, they reinforce each other; when they are of opposite nature, a partial cancellation occurs. The presence of both displacement and velocity responses in the single fibers suggests that outer and inner hair cells of the cochlea interact.*

Transduction of acoustic signals into neural potentials, which takes place in the cochlea of the mammalian auditory system, is effected by two populations of sensory cells—the inner and outer hair cells. There are about three times as many outer hair cells as there are inner hair cells, but about 90 percent of all afferent fibers of the auditory nerve end on the latter (*1*). This means that practically all single-unit recordings from the nerve concern fibers innervating the inner hair cells. The role of the more numerous outer hair cells remains obscure. There is no clear anatomical evidence of interconnections between outer and inner hair cells; however, some structural relationships suggest that one could exist. The number of fibers innervating the outer hair cells appears to be equal to the number of inner hair cells. These fibers course in close proximity of the inner hair cells and join the bundles of fibers innervating these cells. An interaction between the inner and outer hair cells is also suggested by experiments with ototoxic drugs, especially kanamycin. The drug affects the outer hair cells more strongly than the inner hair cells. In the parts of the cochlea where the former are destroyed but the latter appear intact, the sensitivity and frequency selectivity of single units in the auditory nerve are decreased (*2*). Since most of the recordings must refer to fibers ending on inner hair cells, the effect of missing outer hair cells is highly suggestive. Nevertheless, the possibility remains that relevant inner hair cells are also affected in these experiments without evident histological changes.

Experiments of Dallos and his coworkers (*3*) on cochlear microphonics suggest a way of testing the possibility of an inner-outer hair-cell interaction in normal mammalian ears. They found that, in such ears, the cochlear microphonics are approximately proportional to the displacement of the basilar membrane. After kanamycin treatment, the displacement microphonic disappears in the sections of the cochlea where the

outer hair cells are destroyed. The small residual microphonic found in the sections where the inner hair cells are preserved is associated with the motion of the basilar membrane. Because of a possible effect of kanamycin on inner hair cells, the experiment is not conclusive. However, it is sufficient for a working hypothesis that outer hair cells respond to basilar membrane displacement and the inner hair cells to its velocity.

If the outer hair cells respond to displacement and the inner hair cells to velocity, these responses should be reflected in the firing rates of auditory nerve units. Most units should show a velocity response, and a few, a displacement response, if no interaction between the inner and outer hair cells takes place. In the event of such an interaction, both responses could be present in the same fibers.

Using appropriate acoustic stimuli, we have been able to separate the velocity and displacement components of the basilar membrane motion. Such stimuli can be found on the basis of the following considerations. (i) To avoid ambiguities, the fundamental frequency must be low, so that displacement and velocity maxima are separated by long time intervals compared to neural latencies. We used a fundamental frequency of 40 hz. (ii) At low sound frequencies, the displacement of the stapes footplate is in phase with the sound pressure at the eardrum. (iii) The displacement of the basilar membrane at the basal end of the cochlea fundamentally follows the first derivative of the stapes displacement. Dallos (*4*) showed that this may not be quite true at low frequencies, depending on the animal species. However, we have been able to correct approximately for the deviation. (iv) The velocity of wave propagation in the cochlea is practically independent of sound frequency at low frequencies. Therefore, the waveform produced on the basilar membrane near the stapes is reasonably well preserved throughout the cochlea. This permitted us to study units with high as well as reasonably low characteristic frequencies (CF's).

The experiments were performed on tracheotomized Mongolian gerbils (*Meriones unguiculatus*) under sodium pentobarbital anesthesia (35 mg per kilogram of weight) in a double-walled sound- and vibration-proofed booth. Access to the auditory nerve of these animals was gained through the hypertrophied tympanic bulla with only superficial bleeding and without disturbing the sound conducting parts of the middle ear (*5*). When the bulla is opened through a ventrolateral approach, a small air cavity adjacent to the round window membrane becomes visible. The cavity is separated from the auditory nerve by only a thin semitranslucent bony wall. A small opening may be made in this wall so that a microelectrode can be advanced through it under hydraulic control to record from single units within the internal auditory meatus. Glass microelectrodes filled with a 3*M* NaCl solution and with tip resistances of 30 to 70 megohms were used for all recordings. Cochlear microphonic and whole nerve N_1 responses were monitored with a stainless steel electrode placed near the round window. Sound stimuli were delivered to the right ear through a closed acoustic system containing two ½-inch condenser microphones (Brüel & Kjaer) —one serving as a sound source, the other as a monitor. The transducers were coupled to the tightly sealed auditory meatus through two concentric tubes. The acoustic system was calibrated on a 0.1-cm^3 cavity. It produced a frequency-independent sound pressure at the eardrum within the frequency range of interest.

In an initial series of experiments, response latencies to rarefaction clicks were determined as a function of the units' CF. For units with high CF's, the latencies amounted to somewhat less than 1 msec and were shorter than the latency of the peak of the N_1 response. For units with CF's in the neighborhood of 0.3 khz, the latencies increased to about 2 msec. The short latencies indicate that the recorded responses belonged to auditory-nerve fibers (*6*). The same conclusion could

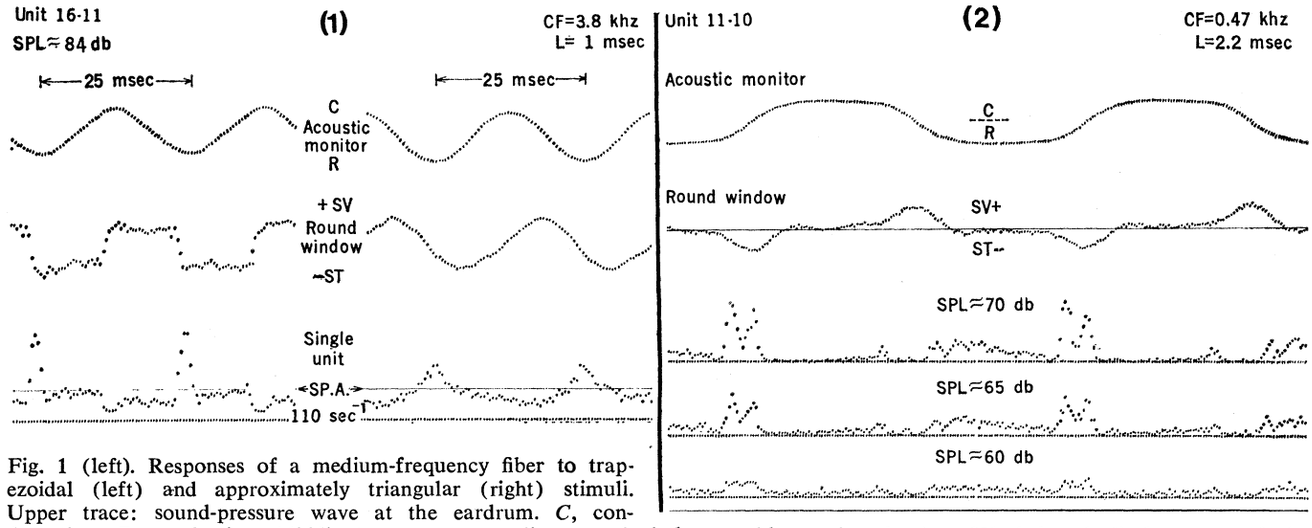

Fig. 1 (left). Responses of a medium-frequency fiber to trapezoidal (left) and approximately triangular (right) stimuli. Upper trace: sound-pressure wave at the eardrum. *C*, condensation; *R*, rarefaction. Middle trace: corresponding round-window cochlear microphonics. *SV*, inferred displacement of the basilar membrane toward scala vestibuli; *ST*, toward tympani. Lower trace: PST histograms (0.5-msec bin width, 2000 repetitions). The thin line marked *SP.A.* indicates the average level of spontaneous activity of approximately 110 spikes per second. Fig. 2 (right). Responses of a low-frequency fiber to positive and negative trapezoidal stimuli. Upper trace: sound-pressure wave at the eardrum. Second trace: corresponding round-window cochlear microphonics. Three lower traces: PST histograms (0.2 msec bin width, 2000 repetitions) recorded at three different SPL's. Symbols and repetition rate as in Fig. 1.

be reached from the predominantly monophasic waveform of the neural spikes (*7*).

To separate the displacement and velocity components in the neural responses, we used the waveforms shown at the top of Figs. 1 and 2. The high frequency spectra of all the waveforms were limited by two electrical filters: the first an LC filter with a cutoff at 1 khz and the second an RC filter with a time constant of 2 msec. The filters prevented ringing in the auditory system (the middle ear and basilar membrane). The second filter also corrected to some extent inferred departures of the basilar membrane displacement from the first-derivative response. The second trace from the top in each figure shows the cochlear microphonics recorded at the round window. Upward deflection means positivity, which is associated with the displacement of the basilar membrane toward scala vestibuli. The bottom two traces of Fig. 1 and the three lowest traces of Fig. 2 show PST (poststimulus time) histograms of responses of a medium frequency and a low frequency unit. These traces were shifted to the left with respect to the cochlear microphonic traces to compensate for neural latencies of 1 and 2 msec, respectively.

Maximum firing rate is associated with the transition from positivity to negativity in the cochlear microphonic (left section of Fig. 1), thus, with the motion of the basilar membrane toward scala tympani; minimum firing rate corresponds to motion in the opposite direction. In addition to these velocity responses, weaker displacement responses are also visible during the motion plateaus indicated by the cochlear microphonic. Displacement toward scala tympani produces a greater firing rate than displacement toward scala vestibuli. However, the level of spontaneous activity is not exceeded, so that the response is mainly inhibitory. To demonstrate more clearly that the firing-rate maxima and minima produced by the trapezoidal left-hand pattern are velocity responses rather than transient displacement responses, the nearly triangular right-hand pattern increases the proportion of the cycle during which motion occurs. Still, the firing-rate maxima are located near the maxima of velocity toward scala tympani; and the firing-rate minima are located near the maxima of velocity toward scala vestibuli. At these points, the displacement is practically zero. The displacement responses are difficult to identify, but they produce an asymmetry in the response pattern. The maximum suppression of firing rate occurs between the maxima of velocity and displacement toward scala vestibuli.

Evidence for a positive displacement response is supplied by the recordings from a low-frequency unit (Fig. 2). The pattern of basilar membrane displacement near the oval window, as indicated by the cochlear-microphonic trace, consists of positive and negative rounded trapezoids spaced by plateaus of nearly zero displacement. Model experiments indicate that, toward the cochlear apex, the plateaus show a somewhat stronger overshoot than is evident in the recording and are tilted toward the zero level. The unit's responses are consistent with this modified pattern. The two prominent peaks of firing rate are associated with the motion of the basilar membrane from approximately zero position toward scala tympani, and with the maximum displacement of the membrane in the same direction. The trapezoidal excursion in the opposite direction produces two regions of minimum firing rate. The long excursion plateau toward scala tympani produces an enhancement of firing rate; the plateau on the side of scala vestibuli, a suppression of firing rate. The velocity and displacement components interact, since intermediate responses result when displacement and velocity are in opposite directions. The small peak that coincides with the motion of the basilar membrane from scala vestibuli toward zero position results from the effect of the excitatory velocity toward scala tympani counteracted by the effect of the inhibitory displacement toward scala vestibuli. To complete the interpretation of Fig. 2, two more features should be pointed out. First, the time interval between the two peaks associated with the trapezoidal excursion toward scala tympani does not coincide either with the natural frequency of the middle ear or with the CF of the unit. Second, the time relationships do not change appreciably with stimulus intensity. This was true from levels that produced just noticeable responses to about 80 db SPL. Experiments on other units showed that the response pattern does not change until very high stimulus levels are reached.

The recordings of Figs. 1 and 2 are consistent with each other and with the results obtained on over 40 other units. They show that nearly all units of the auditory nerve respond to both velocity and displacement of the basilar membrane. According to our hypothesis, this means that the outer and inner hair cells interact. In addition, our results show that motion and displacement toward scala tympani are excitatory, and motion and displacement toward scala vestibuli are inhibitory. This finding contradicts the prevailing belief that displacement toward scala vestibuli produces excitation, but it is in partial agreement with recent results of Konishi and Nielsen (*8*). They plugged the helicotrema with bone wax, drove the cochlea through the round window, and found that most units were excited during displacement of the basilar membrane toward scala tympani. However, most excitatory velocity responses were associated with motion toward scala vestibuli. We found such responses only at very high stimulus levels. Perhaps the unnatural way in which the cochlea was stimulated by Konishi and Nielsen produced the discrepancy. Finally, we should point out the significance of the fact that the velocity as well as the displacement responses can be both excitatory and inhibitory. If the displacement responses are associated with the outer hair cells but the recordings refer to fibers ending on inner hair cells, it is difficult to understand how the outer hair cells can provide excitatory as well as inhibitory inputs to these fibers. Possibly the outer hair cells are responsible for most of the spontaneous activity, as has been indicated by recent kanamycin experiments (*2*). They could inhibit their own spontaneous activity and, in this way, decrease the firing rate in secondary units.

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