NERVE IMPULSES IN INDIVIDUAL AUDITORY NERVE FIBERS OF GUINEA PIG*

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TO UNDERSTAND how the cochlea analyzes and responds to complex sound stimuli, it is desirable to know what kind of message individual nerve fibers carry from the ear to the central nervous system. In 1942, Galambos and and Davis (9) recorded electric responses of single ganglion cells in the cochlear nucleus, which they once believed to be from single auditory nerve fibers. Those responses, recorded from the secondary neurons in the auditory system, indicate that each element responds to a tone of a particular frequency with a particularly high sensitivity. Galambos (8) also described an inhibitory interaction between the responses to two different sound stimuli in the cochlear nucleus. It is therefore possible that the selective response of each element at this level of the auditory system to a particular frequency could be largely due to some complicated interaction among nerve impulses arriving at the cochlear nucleus over a large number of primary auditory nerve fibers.

Quite recently, it has been shown in this Institute (21, 22) that the basal turn of the guinea pig cochlea responds to practically all frequencies in the audible range, while the upper parts of the cochlea respond only to sounds of low frequencies. By the method of differential recording of the microphonic response across the cochlear partition, it was shown that when the response of the upper part of the cochlea (to low-frequency sounds) had been eliminated by a local injection of an isotonic KCl solution there were still good normal responses in the basal turn, both microphonics and nerve action potentials. These results exclude any sharp localization of vibratory motion in the cochlea.

The "resonance curve" obtained by Békésy (3), which correlates mechanical displacement of the cochlear partition of the dead human and animal ears with place in the cochlea at various frequencies, is not very sharp. Nevertheless, the large microphonic and nerve action potentials induced by low-frequency sound in the basal turn still seem difficult to reconcile with Békésy's curves, which show a fairly rapid decay in amplitude of vibration toward the basal turn. More direct information as to the nerve impulses in the primary auditory neurons seemed to offer a solution of this difficulty.

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The discovery of submicroscopic microelectrodes by Ling and Gerard (12) and by Nastuk and Hodgkin (13) opened up the possibility of recording single-fiber responses from small nerve fibers in anatomically restricted positions in the body. The possibility and the difficulties of recording resting and action potentials from nerve fibers have recently been discussed by Woodbury (24) and by Tasaki (19). With these intra-cellular electrodes pushed into the myelin sheath or the axis cylinder, it is possible to record large action potentials which often amount to 30 mV. Actually the attempt to record such single-fiber responses from the auditory nerve turned out to be successful. The results obtained indicated that the nerve fibers arising in the basal turn respond to all the frequencies examined (between 500 and 10,000 cps) at sound levels of about 50 db above 0.0002 microbar. Unlike the cell-body responses from the cochlear nucleus, none of the spontaneous discharges observed in these primary sensory neurons were ever inhibited by sound stimulation.

The synopsis of this work was presented at the Spring Meeting of the American Physiological Society in 1953 (20).

METHODS

Surgical operation. Guinea pigs were anaesthetized with Dial in urethane (0.5 cc./kg. body weight). An incision was made through the skin along the masseter muscle. After ligating the external jugular vein both the masseter muscle and the mandible were cut across in the middle, and the posterior half of the mandible was removed. By lifting the posterior part of the masseter muscle, the bulla was then exposed. Next all the tissues around the styloid process were carefully separated from the surface of the bone and the process was cut across, with a dental drill, as close to its base as possible. After cleaning the surface of the bulla a large opening was made in the bulla to expose the cochlea, the ossicles and the tympanic membrane.

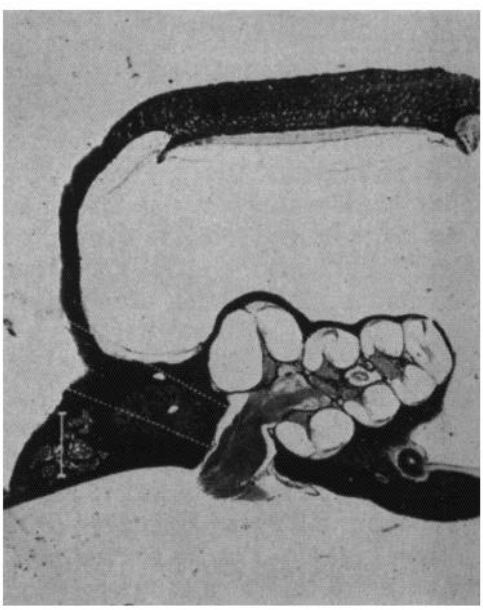

FIG. 1. Mid-modiolar section of guinea pig cochlea. Dotted lines show position, direction and size of hole for approaching nerve fibers in modiolus. Bar at corner subtends 1 mm.

Then, with a small dental drill (size 1/2), a small hole approximately 1 mm. in diameter was made toward the modiolus through the thick bone boundary by the scala tympani of the cochlea, the vestibule and the cerebral cavity. This hole was started on the edge of the bulla at the point approximately 3.5 mm. away from the round window, and at the depth of approximately 2 mm. it reached the modiolus. The histological specimen shown in Fig. 1 shows the position and the direction of the hole. The surface of the eighth nerve was then cleaned with a sharp needle and forceps. All these operations were done under a dissecting binocular microscope of about 10×.

Accumulation of cerebro-spinal fluid in the hole was slow, except when there was a direct communication between this

hole and scala tympani or the cerebral cavity. When the direction of the hole was such that perforation was first made in the wall of the cerebral cavity, the animal was discarded. A direct communication between the hole and the scala tympani did not cause any appreciable loss in the response of the auditory nerve; but, because of rapid accumulation of perilymph in the hole, insertion of the microelectrode into the nerve fibers was considerably more difficult in animals with such a communication.

The head of the animal was fixed to a table provided with three holders made from strong forceps. One holder was used to clamp the zygomatic arch, the second holder applied to the edge of the bulla near the attachment of the eardrum and the third held the upper incisor teeth. If the animal was too active at this stage an additional dose of 0.03–0.05 cc. of Dial was injected intraperitoneally. A proper dose of anaesthetic and frequent heating of the animal (to avoid shivering at room temperature) made the animal quiet enough to permit observation of single-fiber responses with microelectrodes. The external auditory meatus was generally closed with a piece of plasticene, and the sound stimuli were applied through the opening in the bulla.

Electro-acoustic equipment. Sound stimuli used were (i) clicks, (ii) tone pips, (iii) pure sinusoidal waves of various frequencies, and in some cases (iv) sinusoidal waves which were modulated in both frequency and intensity. Clicks were obtained by applying a rectangular voltage pulse to an Atlas PM-25 loudspeaker to which a garden hose of approximately 175 cm. was attached. Tone pips (short bursts of sound waves) were obtained in two ways. Sometimes they were obtained by passing the rectangular voltage pulse through two sets of electric resonant circuits of the desired frequency (see 6). Sometimes pips were obtained by passing a pure sinusoidal wave from an oscillator through an electronic gate designed by Dr. J. Hind (personal communication) and constructed by Mr. H. Ludwig. The pure tones were obtained from a beat-frequency oscillator (General Radio 1304-A or 913-C). In order to make it possible to test the effects of several different frequencies in a short time, the frequency of a beat-frequency oscillator was changed slowly by changing the capacity of the condenser in one of the radio-frequency resonant circuits of the oscillator. The rate of this change was controlled by a motor rotating the plates of the variable condenser. The output of the oscillator was led to the electronic gate which increased the amplitude of the output from the oscillator along an exponential time course. The duration of the bursts of sound and the interval between these relatively long pips were also controlled by the gate. The transducer used for this type of acoustic stimulation was in most cases Signature (Lansing D-175), which has a nearly flat response between 500 and 10,000 cps. The horn of the transducer was directed toward the opening of the bulla at a distance of approximately 50 cm. from the animal. To measure the approximate intensity of the stimuli a dynamic microphone (Western Electric 33A) was placed near the ear of the animal.

Recording electrodes and equipment. Microelectrodes used for recording single fiber responses were made of glass capillaries having a diameter of $\frac{1}{3}$ to $\frac{1}{4}$ μ at the tip. The rate of increase in diameter with distance from the tip was between $\frac{1}{8}$ and $\frac{1}{12}$.* The capillary was filled with a three-molar KCl solution. Electrodes were selected which showed a DC resistance of between 20 and 40 MΩ. The glass capillary was held with a lucite holder and a fine silver wire (0.1 mm. in diameter) connected the capillary to the input of a cathode-follower preamplifier. The output of this preamplifier was amplified with a condenser-coupled amplifier (Grass). The resting potential of the auditory nerve fibers was usually not measured.

When recordings of the cochlear microphonic and whole-nerve action potentials were needed, nichrome-steel wires of about 20 μ diameter were inserted into various turns of the cochlea and, by the method described earlier (21), the various potentials were separated from one another and were recorded simultaneously with independent cathode-ray oscillographs.

RESULTS

1. *Relationship between sound intensity and microphonic responses.* Two pairs of nichrome-steel wire electrodes were inserted into the cochlea, one

* This electron-microscopic measurement was done with the help of Dr. E. Dempsey of the Department of Anatomy, Washington University, to whom I wish to express my gratitude.

pair in the basal turn and the other in the third turn. The microphonic responses from the first and third turns of the cochlea and the sound wave picked up by the microphone near the head of the animal were recorded simultaneously.

An example of the records obtained by this method is shown in Fig. 2. The intensity of the sound field was adjusted at each frequency in order to give a constant voltage across the cochlear partition in the basal turn. As described in an earlier paper (21), the response of the third turn decreased

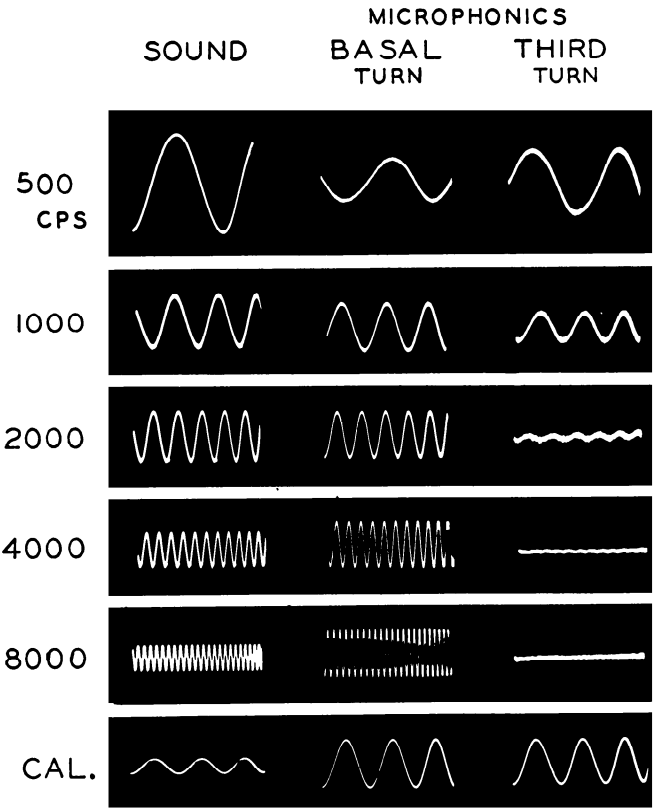

FIG. 2. Relation between intensity of sound (left column) and microphonic responses recorded from basal turn (middle) and from third turn (right) of cochlea. Three records at each frequency were photographed simultaneously. Compare relative amplitudes and phase differences. Calibration at bottom indicates sound pressure of approximately 57 db above 0.0002 microbar at 1000 cps (left) and $\frac{1}{3}$ mV. peak to peak (middle and right).

rapidly as the frequency went up from 500 to 2000 cps. The phase differences between the responses from the two turns in these records agree also with the results described earlier, the difference at 500 cps being approximately $\pi/2$ and at 1000 cps close to π. The level of the sound pressure necessary to produce a constant microphonic potential in the basal turn depended only slightly upon the frequency. At 8000 cps it was about 10 db below that

at 500 cps. In the range of sound intensity employed, the microphonic potential increased linearly with increasing sound intensity.

The difference in the sound levels at high and low frequencies necessary to produce a constant voltage in the basal turn varied greatly with the amount of fluid accumulated around the eardrum. Gradual outflow of perilymph through the four 25 μ holes made in the cochlea for the nichrome-steel electrodes moistened the inner surface of the tympanic membrane. This increased the sound level required, particularly at low frequencies. Moreover, the resonances and anti-resonances in the bulla and also in the closed external auditory canal make accurate comparison of sound level with the microphonic response at different frequencies very difficult.

2. *Shape of single-fiber responses.* The time-course of the single-fiber responses recorded with microelectrodes from the auditory nerve of the guinea pig was not very different from those obtained by the same method from the peripheral nerve of the frog (19). The rising phase of the internally recorded action potential was extremely sharp and the falling phase was of the order of 0.5 msec. (Because of the distortion of the recorded potential by the capacitative current flow through the thin glass wall of the microelectrode, the exact time course of the action potential is not revealed by this method.) The peak value of the observed spike potential was generally between 1 and 10 mV. Sometimes, due to the capacity of the glass wall and the myelin sheath, the falling phase of the positive spike potential was followed by a slight negativity.

On many occasions single-fiber responses were observed before the start of the resting potential (see Records C and D in Fig. 3). This fact, together with the "notches" which often appeared on or near the top of the observed action potential (see the records in the middle column of Fig. 4), indicates that the microelectrode is actually in one of the myelinated fibers in the modiolus. In the frog motor nerve fiber these notches derive from delayed conduction between the nodes of Ranvier on the two ends of the internode punctured with the microelectrode (19). It was also mentioned in the earlier paper that a thin film of myelin, which sometimes covers the tip of the microelectrode, is capable of sustaining the resting potential across it, while the rapid voltage change during activity passes through this capacitative membrane. In several cases the "thin film of myelin" apparently disappeared temporarily after the end of a spike potential (see records in right column of Fig. 4). A resting potential appeared immediately following a response and then gradually returned within less than a second.

The size and the shape of the spike potential changed appreciably from fiber to fiber, and also from time to time in one fiber; but the changes in a given fiber usually progressed very slowly except when a sudden movement of the animal caused a sudden jump of the oscillograph line. As long as the base line of the oscillograph stays quiet, we can be sure that the microelectrode is still recording potentials from the same fiber.

3. *Spontaneous discharges.* In all the experiments described in this paper

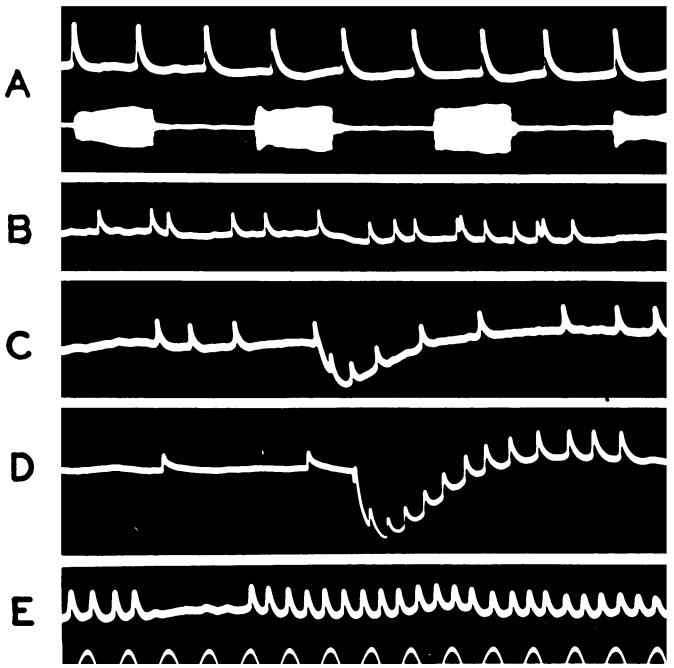

FIG. 3. Discharge of impulses in non-auditory nerve fibers in eighth nerve. A: A regular discharge recorded together with sound stimuli. B: An irregular discharge. C; A low-frequency irregular discharge with start of resting potential in middle. D: Start of an injury discharge. E: An injury discharge. Four different fibers from same eighth nerve. Time marker, 10 msec., applies also to other records. Gradual rise of base-line following start of resting potential in records C and D is due to condenser-coupling in amplifier. In this and all subsequent action-potential records upward deflection represents positivity of the exploring electrode (s) relative to the neck of the animal.

microelectrodes were pushed into the eighth nerve while the sound stimuli (in most cases tone pips of relatively long duration) were being applied to the ear. It was found by this technique that there are in the eighth nerve quite a number of fibers in which the impulse discharge cannot be modified by sound stimuli. These spontaneous discharges can be classified into the following three types: (i) regular spontaneous discharge, (ii) irregular spontaneous discharge, and (iii) injury discharge.

The frequency of regular spontaneous discharges was generally between 30 and 50 per sec. (Fig. 3A). The interval between impulses was more constant when the frequency was higher. This type of spontaneous discharge was slightly less common than the irregular type.

In irregular spontaneous discharges, the interval between the successive impulses in one fiber varied often from 1 to 40 msec. The total number of impulses discharged in a second varied from 10 up to almost 100. The discharge was arbitrarily considered irregular if there was a maximum variation of as much as 3 to 1 in the intervals between impulses. For frequencies of discharge less than 30 per sec., the division between regular and irregular discharges was not clear.

Record B in Fig. 3 is an example of a high-frequency irregular discharge. The shortest intervals between impulses are about 1 msec. Since the absolutely refractory period of a nerve fiber is approximately equal to the spike duration (2, 18), a spike should end always before the start of the next spike; but because of the capacity across the wall of the glass capillary microelectrode, which lengthens the falling phase of the recorded potential, two spikes following closely one after another give a false impression of a summation of two spikes.

An injury discharge is initiated by the appearance of a resting potential in the microelectrode. The frequency at the beginning of the injury discharge may be as high as 300 per sec. or slightly more, but it generally dies away fairly rapidly. This type of discharge usually ceases before its frequency falls below 100 per sec. In fibers in which a low-frequency spontaneous discharge was recorded before the appearance of a resting potential short bursts of (injury) discharges often occurred periodically, apparently following the arrival of each (afferent?) impulse at the site of the injury by the microelectrode.

In Fig. 3C, some of the spikes following the appearance of the resting potential are probably those induced by the injury current, but it is difficult to tell exactly how many of them are due to injury in this case. In Fig. 3D, the spikes induced by injury are slightly larger than the spontaneous impulses recorded before the beginning of the resting potential. Figure 3E shows the progressive change in the size and in the shape of the rising phase of the spikes induced by injury. During the brief cessation of discharge in this record, small elevations are seen which suggest non-propagated responses at the node of Ranvier close to the injured spot. Since, however, a microelectrode can pick up action potentials simultaneously from two different fibers in one nerve trunk (19), the possibility that those elevations are due to impulses in a neighboring fiber cannot be excluded.

The physiological significance of the spontaneous discharges is not clear. It is not possible to distinguish between afferent and efferent impulses by looking at our oscillograms. Spontaneous discharges of impulses were observed in all the animals examined. We identify these impulses as "spontaneous" and not due to injury because they appeared without the appearance of a (DC) resting potential.

It is my impression that most of the non-auditory nerve fibers that carry spontaneous impulses are located in a compact bundle. When the microelectrode is pushed into a particular region in the nerve, the noise level of the oscillograph line goes up and spontaneous discharges which cannot be modified by sounds are recorded from many fibers in this locality. It seems likely that the microelectrode was pushed into the vestibular branch of the eighth nerve in those cases. It is also possible that these fibers belong to the (efferent?) bundle of Rasmussen (14), which is known to survive the degeneration of the afferent fibers which occurs after exposure of the ear to loud sounds.

Spontaneous discharges were present also in auditory nerve fibers which

did respond to sound stimuli. Most of these afferent fibers showed only occasional spontaneous impulses, but in some fibers there were many. In those fibers with a high-frequency spontaneous discharge the effect of an applied sound was often partly obscured by the background activity, but throughout the entire course of the present experiments spontaneous discharges of impulses were *never* inhibited by applied sound stimuli. This is one of the important differences between the primary and secondary neurons in the auditory system.

4. *Responses to short tone pips.* A low-frequency tone pip is the best stimulus for eliciting afferent impulses in the auditory nerve (22). It can excite any auditory nerve fiber if it is strong enough. In response to a 500 cps tone pip two or three impulses usually appeared at the expected interval of approximately 2 msec. Three examples are presented in Fig. 4. In these experiments the microphonic responses of the basal turn and action potentials of the whole nerve were recorded simultaneously with the single-fiber responses. The microphonic and the whole-nerve responses always showed a relatively stable configuration. Especially at high sound intensities most of the responses to those pips, repeated at a rate of 6–8 per sec., looked so similar that only one example of each is presented in the figure. Single-

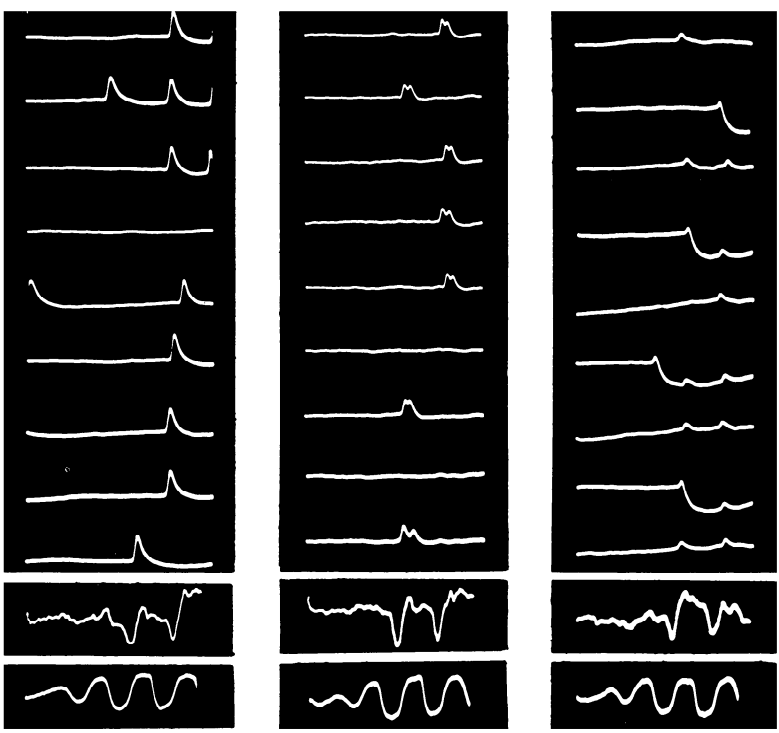

FIG. 4. Responses to strong 500 cps tone pips recorded from three different fibers in one cochlear nerve. Whole-nerve responses and microphonics are shown below. Largest single fiber spikes are of order of 2 mV. and whole-nerve spikes are about 0.1 mV.

fiber responses showed, on the contrary, enormous variations in latency and in the effectiveness of the stimulus. In most cases single-fiber spikes tended to appear at or slightly after the peaks of the whole-nerve responses, but very often they failed to appear at all at the expected moments. Furthermore, when the sound intensity was not very high single-fiber responses appeared only once in response to every two or three pips.

Since all the fibers examined were found to show these erratic responses to a given tone pip, it is clear that the size of a whole-nerve response is determined primarily by the *probability* of the auditory nerve fibers responding to each cycle of the sound wave. The difference in latency among different fibers, however, complicates this kind of argument.

With 1000 cps tone pips single-fiber responses also showed a definite tendency to appear at the frequency of the applied sound waves. Here again many of the single-fiber spikes were seen to start at or shortly after the peak of each whole-nerve response. The probability of obtaining a spike at this moment increased directly with the size of the observed whole-nerve response. It has been shown previously (5) that at this frequency the whole-nerve responses tend to appear at the frequency of the applied sound.

Strong high-frequency tone pips are adequate for measuring the time interval between the start of the microphonic response and the start of the whole-nerve response (5, 10). With strong 8000 cps tone pips, it was found that the latency of single-fiber responses was not constant but varied considerably from pip to pip (pips repeated at a rate of 7–8 per sec.). A fiber which has responded to a pip with double spikes at a relatively short latency may respond to the next pip of the same intensity with a single spike at a long latency or even with no spike at all (Fig. 5). The whole-nerve responses, which were recorded with a pair of small metal wire electrodes inserted in the basal turn, showed practically no fluctuation of amplitude or latency at this intensity.

The time interval between the start of the microphonic response and the earliest single-fiber response measured with strong 8000 cps tone pips varied among different fibers from 1.1 to 1.3 msec. The latency for the peak of the whole-nerve response at this sound level was very close to 1 msec. (10).

At 8000 cps it is impossible for individual fibers to follow the frequency of the applied sound because of the refractoriness of the fibers. It is known that such a high-frequency pip induces generally two, sometimes three, whole-nerve spikes approximately 1 msec. apart (5) (see Figs. 5, 6). Single-fiber responses were found to show, as in the case of low-frequency tone pips, a tendency to appear at the peak or in the falling phase of the whole-nerve spikes. The variability in the latency was, however, far greater with these high-frequency pips than with low-frequency pips; this accounts for the fact that a whole-nerve response to an 8000 cps pip shows less steep rising and falling phases than a response to a 500 cps pip.

The second peak in the whole-nerve response to an 8000 cps tone pip is called the "N_2-response," and has often been attributed to the secondary

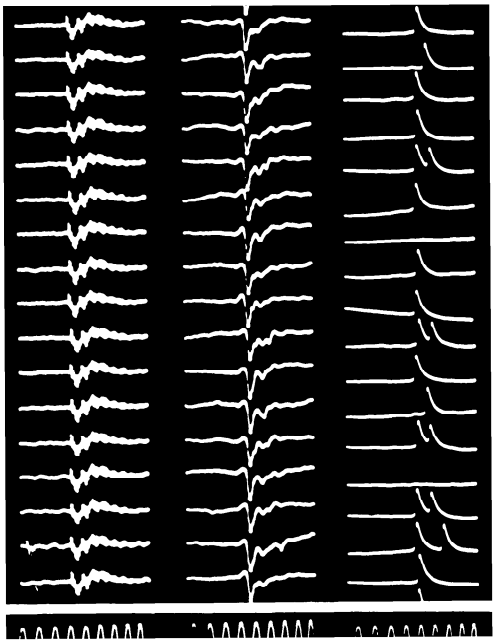

FIG. 5. Microphonics (left), whole-nerve action potentials (middle) and single-fiber responses (right) induced by 8000 cps tone pips repeated at 8 per sec. Amplitude of microphonic responses was approximately 0.1 mV. (peak to peak), whole-nerve responses about 0.25 mV. and single-fiber spikes about 2.5 mV. Time marker, 1 msec.

neurons in the cochlear nucleus. But the primary neurons in the auditory system tend to respond to an 8000 cps pip with double, sometimes triple, spikes; and our records show a parallelism between the size of the N_2-response from the whole-nerve and the probability of obtaining double responses from single fibers. In the experiment of Fig. 5, for example, the N_2-response was small, and double responses were observed in only five cases out of 17 successive stimulations. (Note also the variation in the latency of the second spike.) In the example of Fig. 6, the N_2-response was large and repetitive discharges were very frequently observed. The major portion of the N_2-response therefore seems to represent the repetitive responses in the primary neurons, and not responses in the secondary neurons.

A few records were made of single-fiber responses to clicks. The loudspeaker used for this purpose (Atlas PM-25 coupled to 175 cm. of garden hose) showed a sharp resonance at approximately 8000 cps. The shortest latency of the single-fiber responses to this click did not seem to differ from that to an 8000 cps pip.

5. *Response to pure tones.* On the individual cell bodies in the cochlear nucleus, Galambos and Davis (9) investigated the phase relation between the sound wave and the responses of single elements. We have made similar observations on individual cochlear nerve fibers at several different frequencies. Two examples of the records obtained at 1000 cps are presented in Fig. 7. In these experiments the sound wave (picked up with a dynamic microphone) and the single-fiber responses were recorded simultaneously with two oscillographs. The oscillograph lines were triggered by the output of the audio-oscillator driving the loudspeaker, thus giving a standing picture of two or three full cycles of the sound wave on the oscillograph screen. The intensity of the sound was approximately 40–55 db above the normal human threshold. The microelectrode was pushed into the auditory nerve while the sound wave was being applied through the opening of the bulla of the animal.

For frequencies lower than 2000 cps it was possible to record single-fiber

responses which tended to appear at approximately the same point in the cycles of the stimulating sound. In other words, the interval between these spikes showed a tendency to be some integral multiple of the period of the applied sound wave. There were also many cases in which this tendency was not clear at all. In such cases the microelectrode was probably pushed into non-auditory nerve fibers or, in some other cases, spontaneous afferent impulses may have masked the impulses induced by the sound stimuli.

For consideration of the initiation of nerve impulses in the cochlea the relationship between the phase of the cochlear microphonic and the start of the single-fiber spikes is important. This was investigated by recording the microphonic responses of the basal turn together with the single-fiber responses. The stimuli were not simple sinusoidal waves; instead, their amplitude was slowly increased by means of the electronic gate. This was done to increase the probability of getting a response in one cycle by avoiding adaptation (which progressively decreases this probability during the action of a continuous sound wave). The sweep of the oscillograph beams was triggered by the output of the audio-oscillator ahead of the amplitude modulation by the gate, giving on the screens of the oscillographs a standing sinusoidal wave of slowly increasing amplitude. In order to separate the individual sweeps, the figures on the screen were photographed with a running film.

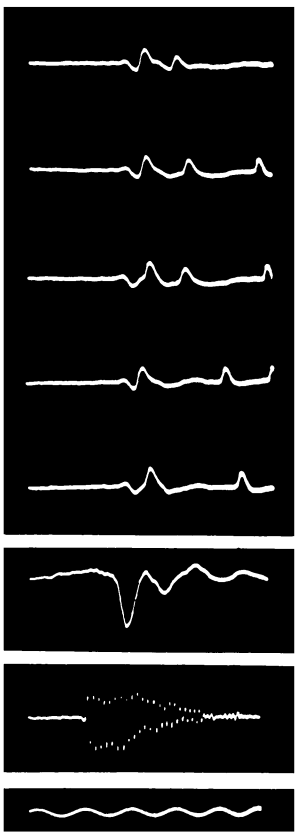

FIG. 6. Same as in Fig. 5, but with longer pips. Distortion of base-line immediately before first single-fiber spikes is due to whole-nerve responses picked up by microelectrode. Time, 1 msec.

At a frequency of about 290 cps single-fiber spikes appeared, in all of the six cases examined, toward the end of the negative phase of the microphonic wave (*i.e.*, while the potential in scala vestibuli of the basal turn still was lower than that in scala tympani). In other words, single-fiber spikes recorded at the position of the hole in Fig. 1 tended to appear during the period between $-\pi/4$ and zero of the sinusoid of the microphonic wave recorded from scala vestibuli. The start of the spikes depended slightly on the intensity of the sound: the higher the intensity the shorter was the latency. But this shift by a gradual change in the intensity was generally within the range of $\pi/4$ radian at this low frequency (see Fig. 8). Similar observations were made at frequency of 500 cps. Out of 23 different fibers on three different animals 12 fibers showed a tendency to respond between 0 and $\pi/2$ (namely, in the positive rising phase of the microphonic response in scala vestibuli of the basal turn), three fibers between $\pi/2$ and π, three fibers

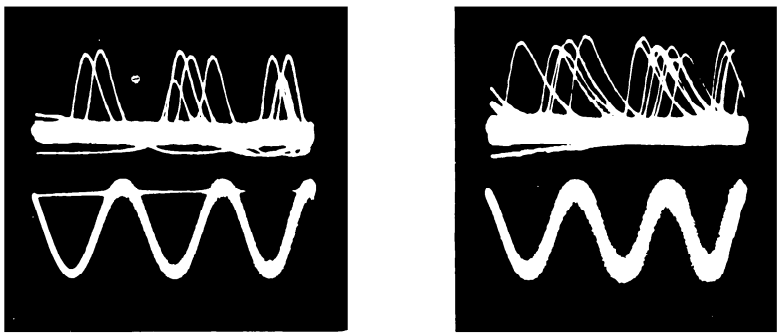

FIG. 7. Single-fiber spikes induced by 1000 cps pure tones. Sound intensity was approximately 55 db above normal human threshold. Sinusoidal standing wave shows sound stimulus recorded with dynamic microphone. Figure on oscillograph screen was photographed with a stationary film; exposure approximately 0.5 sec.

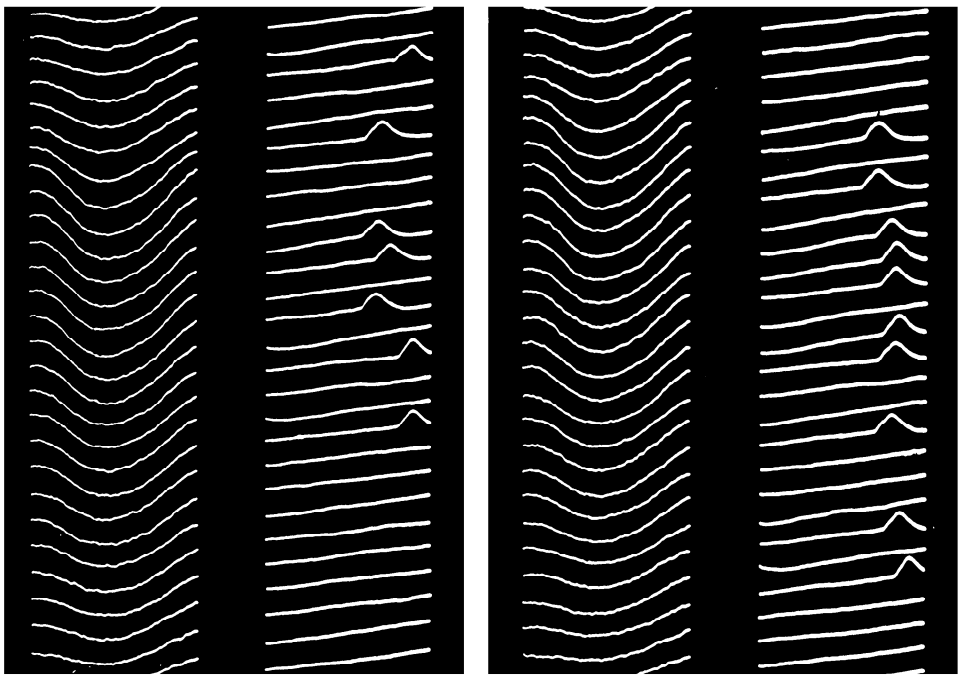

FIG. 8. Time relation between microphonic responses (records on left in each column) and single-fiber responses (right). Sound stimuli were slowly increasing 290 cps tones of approximately 50 db above normal human threshold in room. Deflection of left oscillograph beam was upward when scala vestibuli became positive relative to scala tympani. Maximum amplitude of observed microphonic response is approximately 0.1 mV. peak to peak. The two columns were taken from same fiber. Continuous recording, starting at bottom of figure.

between π and $3\pi/2$ and the remaining five fibers between $3\pi/2$ and 0 (very close to zero in three cases).

A few trials were made with 2000 cps tones. Although there was some tendency for the impulses to start at a definite phase of the sound wave, the results were not very clear because, at this frequency, one cycle of the

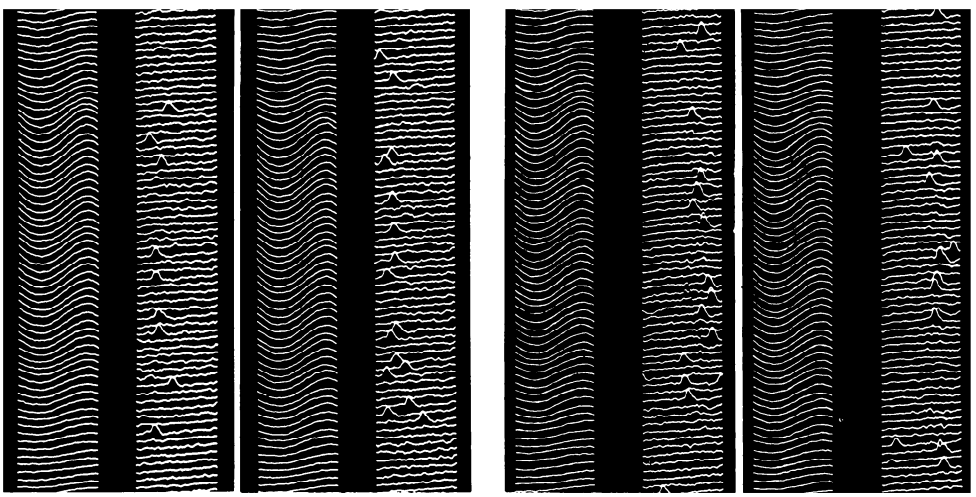

FIG. 9. Same as in Fig. 8, but with 500 cps tones. Right and left sets of records were taken from different fibers in same cochlear nerve. Sound intensity in room was approximately 40 db above human threshold.

sound wave was too short as compared with the variability in latency of the spikes. The experiment was not tried at 4000 or 8000 cps.

6. *Nerve impulses starting in different parts of cochlea.* In this series of experiments the sound stimuli were a train of tone pips the frequency of which was changed gradually from approximately 500 to 9000 cps. The pips were repeated at a rate of 7–20 per sec. Within a pip the amplitude of the modulated wave was increased and decreased by means of the electronic gate approximately exponentially, the time constant of the amplitude increase being in most cases about 15 msec. and that of the amplitude decrease about 5 msec. The intensity of the pips was in general 50–70 db above the human threshold in the room.

In Fig. 10 are presented responses of four different nerve fibers in the modiolus obtained from one and the same animal under constant experimental conditions. In many fibers responses to sound stimuli were found to appear in a limited range of frequency, limited only on the high-frequency side in the range employed. From this one animal responses from 30 different fibers were recorded. Among those 30 fibers, 12 fibers responded to all frequencies between 500 and 8000 cps, nine fibers showed an upper limiting frequency between 4000 and 7000 cps, seven fibers between 3000 and 1000 cps, and the remaining two fibers had their limiting frequencies between

500 and 1000 cps. At this intensity of stimulating sound no fiber was encountered for which the response was limited on both the lower and the higher sides. This type of experiment was done on more than 150 different fibers from eight successfully operated animals with pips of different shapes and different repetition rates. In all the animals except the one mentioned above more than 50 per cent of the fibers examined were found to respond to any

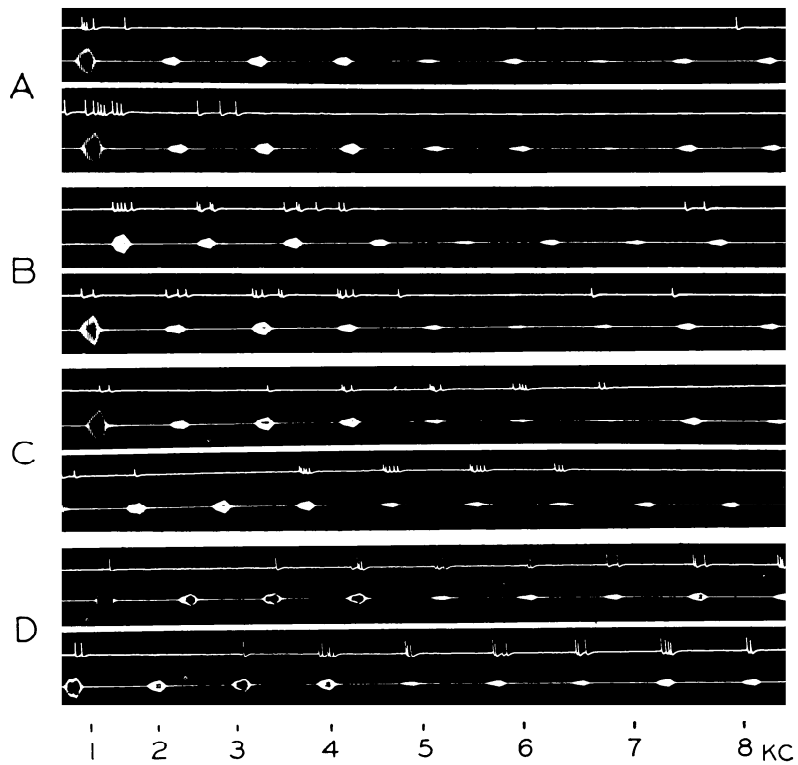

FIG. 10. Responses in four different fibers in a cochlear nerve to tone pips of between 600 and 8300 cps. Sound stimuli were recorded simultaneously by means of microphone. Interval between pips was approximately 50 msec. Sound pressure for pip at resonance point near 1000 cps was approximately 94 db above 0.0002 microbar. Microphone used was almost flat from 500 up to 9000 cps. Spike-height of largest single-fiber response was about 3 mV.

frequency between 500 and 8000 cps. In one particular animal, all of the 26 fibers examined showed unmistakable responses to all the frequencies employed.

There is no doubt that the basal turn is the only place in the cochlea which responds to high-frequency tones (cf. 21). Whole-nerve action potentials induced by a high-frequency tone pip are modified strongly by application of KCl or polarizing current even when the action is limited to the basal turn (22). We should therefore ascribe *all* the responses to high-frequency

sounds (above, say, 7000 cps) to fibers arising in the basal turn of the cochlea. But those same fibers were found to respond also to low-frequency tone pips (see fiber D in Fig. 10).

Recent experiments by Békésy (3) and a series of experiments from this Institute (21, 22) have proved beyond any doubt that the apical part of the cochlea responds only to low-frequency tones. In a preliminary experiment, it was demonstrated that when the train of tone pips (used in the experiment of Fig. 10) was applied to the cochlea, only the tone pips of below 2000 cps generated microphonic responses in the apical one-fifth of the cochlea. Thus it is clear that those fibers for which the responses are limited to the low-frequency range (fiber A in Fig. 10) must arise in the apical part of the cochlea. It follows, from the same type of argument, that those fibers having their limiting frequencies in the middle-frequency range (fibers B and C) originate from the nerve endings in the middle part of the cochlea. The limiting frequency of a nerve fiber never changed during the course of an experiment. As we shall see later, it depends only slightly on the intensity of the tones.

7. *Threshold intensity as a function of frequency.* For secondary neurons, Galambos and Davis (9) were able to measure the threshold intensity as a function of frequency. The direct method used by those authors, however, did not seem to be applicable to the nerve fibers in the modiolus. Because of the highly variable character of the single-fiber responses, many tests are needed to determine the threshold for even one frequency; and it is difficult to keep a microelectrode in one nerve fiber for a period longer than about 10 sec.

One method tested in an attempt to measure threshold for a single-nerve fiber at different frequencies was to use a series of pips of fixed frequency but slowly increasing intensity, and to determine the sound pressure when the first responses occurred. It was expected that a fiber would respond every time as soon as a certain threshold intensity had been reached. But actual trials showed that this method is entirely inadequate because of the great variability in the intensity at which the first response appeared. A better method was, using the arrangements for the experiment of Fig. 10, to change the intensity level by steps and to examine the whole pattern of responses to pips of varying frequency. On a few favorable occasions it was possible to record responses from the same fiber for a period longer than 10 sec. This period was long enough to record the responses to tone pips of about ten different frequencies at several different intensity levels, because the time required for the automatically increasing frequency to change from 500 to 9000 cps was in general only 0.5–0.7 sec. This method showed that at low intensities some fibers respond to tones in a limited frequency range, limited on both the high- and the low-frequency sides. The upper frequency limit was not influenced appreciably by the sound intensity, but the lower limit depended strongly upon the intensity level of the tones.

The dotted curve shown in Fig. 11 was constructed from the action

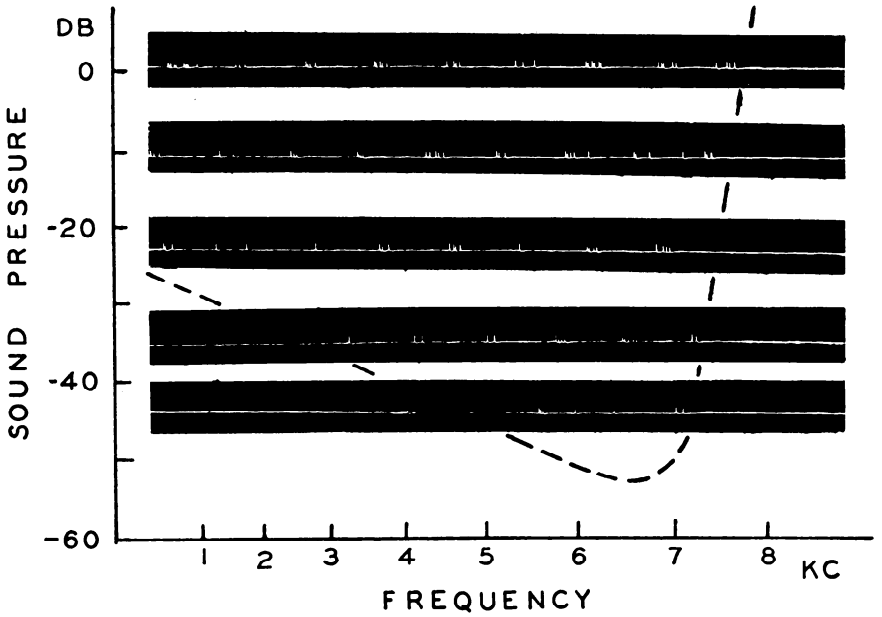

FIG. 11. Responses of a single auditory nerve fiber to tone pips of different frequencies and intensities. Stimuli were similar to those used in experiments of Fig. 10, but tone pips for uppermost records (0 db) were 10 db stronger than in previous experiment. Dotted line shows boundary of response area of this fiber.

potential records of a single fiber in which a microelectrode stayed for more than 100 sec., by connecting the frequency limits at different sound levels. This curve represents the boundary of the "response-area" (see 9) and expresses the relationship between the frequency and intensity of pips which just excited this particular nerve fiber.

The extreme steepness of the curve on the high-frequency side is certainly comparable to the corresponding part of Galambos and Davis's curves for the secondary neuron. It indicates without doubt that at a given spot in the cochlea the mechanical vibration of the cochlear partition becomes suddenly very small when the frequency is increased above a certain limit which is characteristic of the position in the cochlea. The presence of such a "cut-off frequency" at each place in the cochlea has been demonstrated by Békésy (3) who observed the mechanical displacement of the partition (on dead animals) and also by Tasaki *et al.* (21) who measured the cochlear microphonics at different places in the cochlea.

The gradual change in threshold on the low-frequency side of the curve in Fig. 11 is very different from the corresponding part of the curve obtained for the cochlear nucleus by Galambos and Davis (9). The process of inhibition, which has been demonstrated by Galambos (8) in the cochlear nucleus, seems to be the main cause of the difference between the response areas of the primary and the secondary neurons. The inhbition in question must occur in the cochlear nucleus because, as noted in Section 3, we never

detected any indication of inhibition by tones or pips of the spontaneous discharge in primary neurons of the auditory system.

It should be pointed out in this connection that the slope of the curve in Fig. 11 depends to some extent on the method of presenting the sound stimuli to the animal. The dynamic microphone placed near the head of the animal measured the sound pressure in the field to which the animal was exposed, but the actual pressure which drives the oval window is modified by the resonance of the bulla and by the dynamics of the middle ear. Accumulation of a small amount of fluid on the inner side of the tympanic membrane can raise the threshold for low-frequency sounds more than 20 db without affecting the threshold for high frequencies. In spite of these complications, however, it seems safe to conclude that the curve relating frequency and threshold intensity for an auditory nerve fiber in the modiolus consists of two parts, one a gradual fall in threshold with increasing frequency and the other a sharp rise in threshold for frequencies above a certain frequency. The frequency at which the threshold shows a rough minimum is a function of the position of the nerve endings in the cochlea.

According to the results of measurements of the amplitude of the microphonic responses as a function of frequency at different places in the cochlea (21), this position of the minimum threshold for a given frequency should be in the region of the cochlea where the vibration of the cochlear partition lags behind that near the round window by 0.5–1.0 cycle of the sinusoidal wave. In a series of experiments similar to that of Fig. 2, it has been shown that near the frequency at which the microphonic response from an upper part of the cochlea lags behind that from the round window by one full cycle (approximately 1 kc. at the third turn, as can be seen in the figure) the amplitude of the microphonic response from the upper part begins to fall off rapidly with increasing frequency.

In the experiment of Fig. 2 the sound pressure was decreased gradually with increasing frequency in order to keep the basal turn microphonic at a constant level. If, on the contrary, the sound pressure outside the animal is held constant at different frequencies, as it was approximately in all other experiments described in this paper, the microphonic responses for high-frequency tones should be slightly accentuated on the high-frequency side. In other words, under the conditions of the present experiments the microphonic response at a given spot in the cochlea increases gradually with increasing frequency, reaches a rough maximum, and finally, at the cut-off frequency, begins to fall off rapidly as the frequency is increased further.

The threshold-frequency curve, therefore, reflects directly the size of the microphonic response at each frequency. This conclusion gives a strong support to the view that the microphonic responses are the direct stimulating agent for the auditory nerve endings (23, 7). Considering the results of Békésy's direct observation on the displacement of the cochlear partition (3), it might be inferred further that in the basal turn low-frequency tones cause very small displacements, far smaller than in the upper turns, but

the excitatory effect of this small displacement upon the hair cells and the nerve endings is only slightly smaller than its effects in the upper part of the cochlea.

8. *High- and low-threshold fibers.* During the course of experiments with tone pips of changing frequency, it was sometimes noticed that some fibers respond to a pip with a very small number of afferent impulses while in

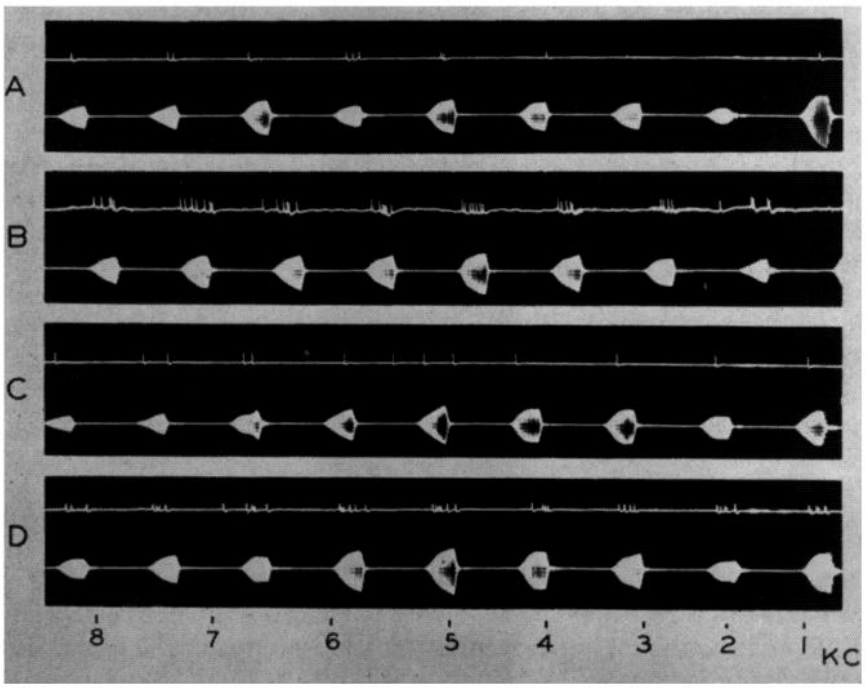

FIG. 12. High-threshold (A and C) and low-threshold (B and D) responses in four different basal-turn fibers in same cochlear nerve under constant experimental conditions. Sound intensity for 4000 cps pip was approximately 105 db above 0.0002 microbar. Pips were repeated at approximately 50 msec. intervals.

many other fibers the same pip elicits a large number of spikes. An example of such results is presented in Fig. 12. All these records were taken from one and the same animal under constant experimental conditions. In this particular animal all of the 26 different auditory nerve fibers examined responded to all the frequencies between 500 and 9000 cps at the sound level of 80–105 db above 0.0002 microbar. The recording microelectrode was apparently always pushed into the bundle of nerve fibers arising in the basal turn. Among 26 fibers, eight fibers responded with an average number of 0.8–2.0 impulses to a pip, one fiber with 3.5 impulses per pip and the remaining 17 fibers responded with 4.0–6.2 impulses per pip. This result suggests that fibers from the basal turn consist of two types, one with high threshold and the other with low threshold. The difference in excitability among these fibers is not due to the variation in the condition of the fiber

because the average number of impulses in response to a pip did not change as long as a microelectrode stayed in the same fiber. Fibers with different excitability were encountered during the course of an experiment in random order. This excludes the possibility that the difference in excitability was due to a progressive change in the condition of the animal.

A very reasonable explanation for this difference in response, suggested by Dr. Hallowell Davis, is as follows: The low-threshold fibers may be those arising from the external hair cells and the high-threshold fibers those innervating the internal hair cells. It is known (see 4) that exposure of the guinea pig ear to a sound of an appropriate high intensity destroys only the external hair cells, leaving the internal hair cells almost intact. It therefore seems that the external hair cells are exposed to a greater vibratory motion than the internal hair cells. The ratio of the number of nerve fibers innervating the internal hair cells to those for the external hair cells is not known at present.

For the fibers arising in the upper parts of the cochlea the distinction between the high- and low-threshold fibers was not very clear due to the difficulty in selecting a proper sound level to demonstrate the difference in excitability by counting the number of impulses. Unless the sound intensity is adjusted to the threshold for the fibers with lower excitability, it is difficult to demonstrate the difference clearly. And for the fibers coming from the upper part of the cochlea the cut-off frequency greatly limited the number of observations. It is my impression, however, that a great difference in excitability exists also among the nerve fibers arising in the upper parts of the cochlea.

The number of impulses induced by a tone pip increased with increasing intensity of sound, but this dependence was less marked at higher intensities. The number of impulses increased approximately logarithmically as the sound pressure. This logarithmic law, which Adrian described in 1928 (1), has, however, only a statistical meaning in the auditory system. Almost all the afferent fibers show a tendency to discharge impulses spontaneously in absence of any sound wave, indicating that the process underlying initiation of impulses at the nerve endings is fluctuating spontaneously without any external stimulus. Apparently due to this spontaneous variation in excitability at the nerve endings, discharge of impulses by a train of tone pips of a constant intensity and a constant frequency fluctuates from time to time in an unpredictable manner. (See the lower record in Fig. 13B.)

Figure 14 gives an example of this statistical logarithmic law of discharge in relation to the sound intensity. In this type of experiment the intensity of the tone pips (either 500 or 5000 cps) was changed at a speed far slower than the rate of rise and fall of intensity in each pip. The thick line in the figure was drawn in accordance with the formula

$$N = D(1/k) \log_e (I/I_0),$$

where N signifies the number of impulses induced by a pip of the duration

D and intensity I, and k and I_0 are the constants to be chosen to fit the curve. This result suggests, on the basis of the arguments stated elsewhere (17, 16), that the frequency of impulses initiated at the endings is determined by the relative intensity I/I_0 of the stimulus and by the recovery from the refractoriness at the initial part of the afferent fiber. Then the recovery of excitability (reciprocal of threshold) should be given roughly by the function $e^{k/t}$ (t is time and e the base of natural logarithms). The value of k was between 5 and 6 msec.

At 500 cps also, the observed relation between the number of impulses and the intensity of sound seemed to obey the logarithmic law, but the result was less clear at this frequency than at 5000 cps. Due to technical difficulties other properties of the fibers used for this type of experiment (*e.g.*, their origin in the cochlea and their sensitivity to sound) were not examined.

9. *Adaptation.* Since the time of Adrian's pioneer work (1), it is well known that, in many sensory nerve endings under the action of a constant sensory stimulus, the frequency of impulses in an individual fiber decreases progressively with time. In individual auditory nerve fibers this gradual decrease in the frequency of impulse was regularly observed during the action of a sound stimulus of a constant frequency and intensity. It was my impression that process of adaptation was more marked at higher frequencies and at higher intensities. But no systematic investigation was made on these problems.

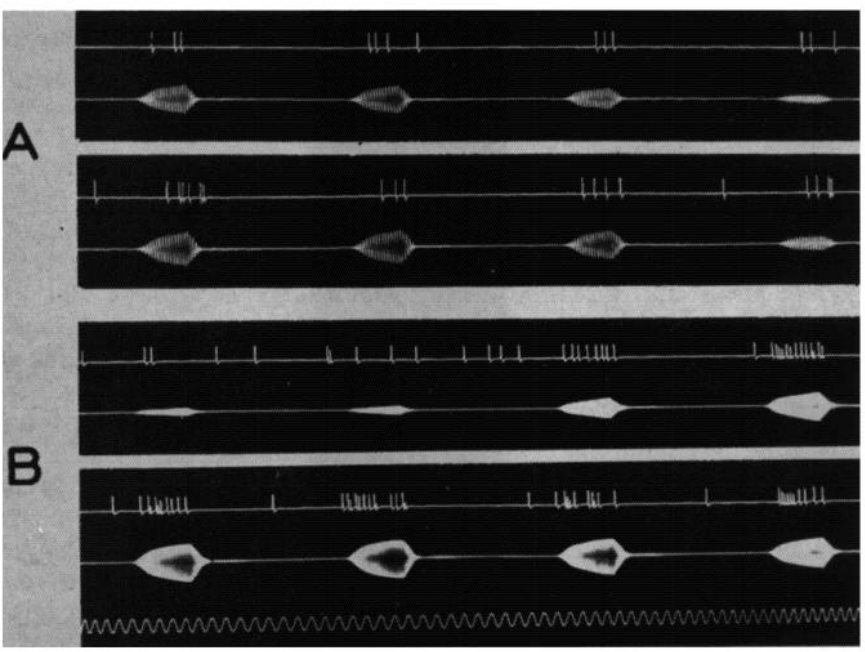

FIG. 13. Effect of sound intensity upon number of impulses. A: responses to 500 cps tone pips; strongest pips in picture were approximately 104 db above 0.0002 microbar. B: Responses to 5000 cps tone pips; strongest pips were approximately 106 db. A and B were taken from two different fibers in same cochlear nerve. Time marker, 10 msec.

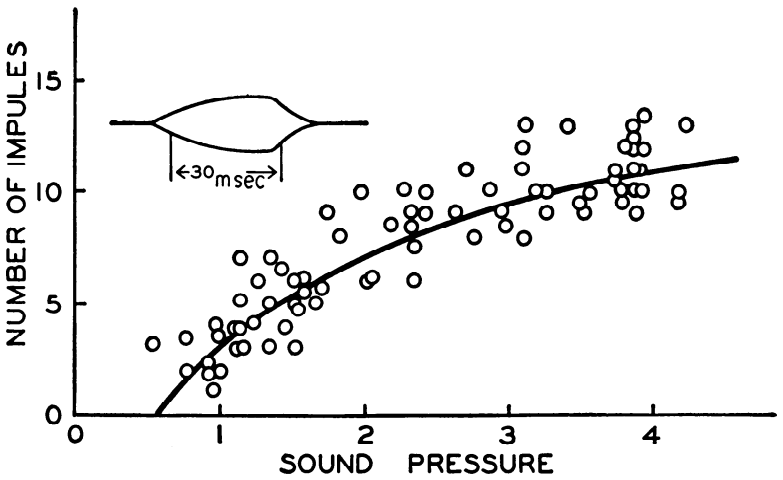

FIG. 14. Relation between intensity of a pip and number of impulses elicited by pip in a single auditory nerve fiber. Frequency, 5000 cps. Abscissa represents sound pressure in microbars (linear scale). Shape of pips is given.

10. *After-discharge.* When a relatively strong tone pip was applied to the ear the discharge of impulses often continued for a short period (sometimes longer than 30 msec.) after the end of the pip. Figure 15 shows an example. It is unlikely that this after-discharge is due to stimulation by echos in the room (which might persist for a short time after the end of a pip), because the sound level measured with a dynamic microphone placed near the head of the animal was, toward the end of the after-discharge, more than 50 db below the intensity of the pip. Since the microphonic response recorded from the cochlea never persists more than 1–2 msec. after the end of the applied sound, the after-discharge is probably due to a persisting activity at the nerve endings. The irregular nature of the after-discharge seems to exclude the possibility that it might be due to an injury either at the region of the nerve endings or at the recording microelectrode.

DISCUSSION

Many of the implications of the results obtained by recording single-fiber responses from the cochlear nerve have been pointed out in the preceding sections. However, the finding that the auditory nerve fibers arising in the basal turn respond to sounds of any audible frequency deserves further comment.

In previous papers (22, Fig. 4; 21, Fig. 4) it has been shown that a small amount of KCl solution introduced into the apex of the guinea pig cochlea eliminates the microphonic responses (to a low-frequency pip) from the apical region without appreciably affecting the size of the whole-nerve action potentials. A similar treatment of the basal turn with KCl eliminated the major portion of the nerve responses. Apparently the impulses traveling along the fibers from the upper part of the cochlea constitute only a small part of the whole-nerve response. The impulses in those fibers are asynchro-

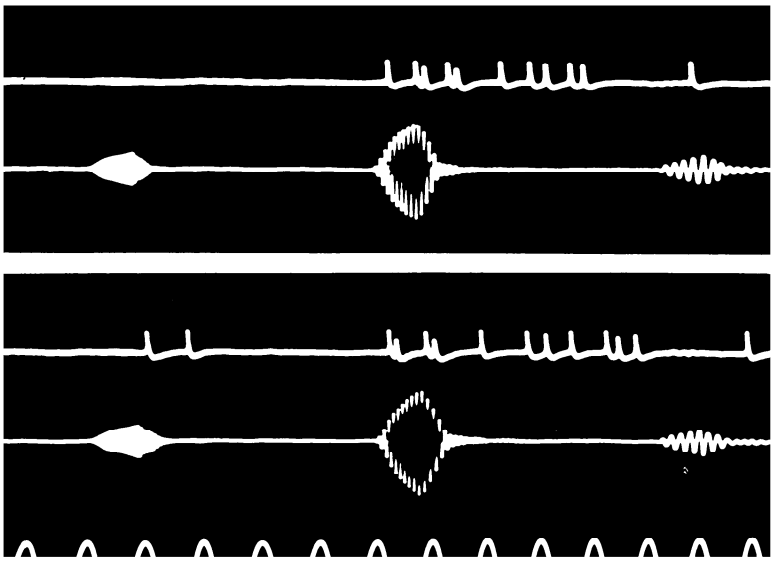

FIG. 15. After-discharge of impulses following stimulation with a strong pip. Time marker, 10 msec. Intensity of pip in middle of figure (1100 cps) was approximately 94 db above 0.0002 microbar (maximum).

nous (except at extremely low frequencies) because of the traveling wave pattern of the basilar membrane, and the number of such fibers is only a small fraction of the total auditory nerve fibers. Thus all the previous experiments in which microphonic responses and action potentials were recorded with electrodes placed near or in the basal turn of the cochlea must now be considered as giving information as to the function of the basal turn only and not the upper part of the cochlea.

The fact that the responses of the basal turn fibers appear at or slightly after the negative peak of the whole-nerve response (Figs. 5, 6) supports the view (7) that the whole-nerve response is generated by the nerve impulses traveling in the modiolus. The distance from the spiral ganglion in the basal turn to the internal auditory meatus is something like 2 mm., and the rate of conduction in this part of the auditory nerve fiber should be, on the basis of the fiber diameters, about 10 m./sec. The recording microelectrodes were pushed into the fibers at a point approximately half way between the spiral ganglia and the internal auditory meatus. It has been shown previously (7) that the potential in the modiolus (recorded with a gross electrode) shows a sharp negativity at or very slightly after the negative peak of the ordinary whole-nerve response.

The experimental results obtained with microelectrodes shed some light on the problem of the initiation of auditory nerve impulses at the nerve endings. At 290 cps single-fiber spikes appeared toward the end of the negative phase of the microphonic wave. Allowing approximately 1 msec. for the conduction from the endings to the recording microelectrode (cf. 7), it is

found that the impulses must have arisen at the endings slightly before the peak of the negative phase of the microphonic wave (recorded from scala vestibuli of the basal turn). In other words, afferent impulses are initiated in the period when the electric current (which generates the observed microphonic potential) is flowing through the hair cells from scala vestibuli to scala tympani. (Note that in a continuous conducting medium a negative potential appears when there is a sink of current in the neighborhood.) Furthermore, a direct current applied across the cochlear partition flowing from scala vestibuli toward scala tympani enhances the nerve responses (22). This gives strong additional experimental support to the view that the microphonics are the internal stimulus which excites the afferent nerve endings electrically.

The results obtained with 500 cps tones are complicated slightly by the fact that single-fiber spikes from different fibers appear in different phases of the cycle of the microphonic response. But the reason for such variety in the phase at which single-fiber spikes appear is clear. It has been shown (21) that at 500 cps the phase of the microphonic response recorded from the apical quarter (namely, the third and fourth turns) of the guinea pig cochlea lags behind that of the response of the basal turn by more than π radians. Since these microphonic responses are the sign of mechanical movement of the cochlear partition in response to the applied sound wave, and since they are directly connected with the process of initiation of nerve impulses at the nerve endings (23, 7), we should expect the impulses initiated in the third or apical turn to lag behind those of the basal turn fibers by more than π radians. The difference in conduction distance (from the end-organs to the site of recording) among those nerve fibers could also give rise to some difference in the time of arrival of those impulses at the site of the microelectrode, but because of the anatomical arrangement of the guinea pig cochlea this difference must be small.

At 500 cps the basal half of the cochlea vibrates almost simultaneously so that the difference in phase of the motion is less than $\pi/4$ radian (21). Since the number of afferent fibers per unit length originating in different parts in the cochlea does not differ appreciably from turn to turn (11, for the human ear; Fernández, unpublished, in the guinea pig ear), the majority of the cochlear nerve fibers should respond in a definite phase of the basal-turn microphonic. Actually, those synchronized nerve impulses gave rise to well-defined whole-nerve action potentials (see examples in Fig. 4). The majority of the single-fiber spikes (recorded in the modiolus) tended to appear at the phase 0 (at which the whole-nerve response shows a sharp peak) or slightly later. It is therefore quite safe to conclude that those fibers which responded in the period between 0 and $\pi/2$ in phase angle originate in the basal half of the cochlea. And again, allowing 1 msec. for the conduction between the nerve endings in the basal turn and the site of recording, it is found that the nerve impulses are initiated at the endings in the early half of the negative phase (scala vestibuli negative to scala tympani) of the

microphonic wave. This agrees with the conclusion drawn from the results for 290 cps.

At 290 cps the time required for the mechanical wave to travel along the cochlear partition towards the apex is much shorter than at 500 cps. The phase difference of the microphonic waves is not more than $\pi/2$ between the two extreme ends of the cochlear partition. In other words, the entire cochlear partition is beating almost synchronously at this low frequency. One should therefore expect all the single-fiber spikes to appear in the same relation to the microphonic wave. Our observation showed that this is actually the case.

Taking whole-nerve responses as their index, Rosenblith and Rosenzweig (15) arrived at the conclusion that the nerve impulses start at a definite phase of the microphonic wave. As has been pointed out above, their experiments deal only with the responses of the basal turn fibers and the microphonic responses in the basal turn. The presence of such relationship between these two types of responses is therefore in good agreement with our results.

The cochlea has long been considered as a kind of wave analyzer which is capable of separating a compound sound wave into its components. This notion is, however, only partly true. In the basal turn a mixture of high and low tones causes a mechanical vibration *as such*, namely, without its being resolved into its components, and excites the nerve endings in the form of the applied mixed wave. Separation between the components occurs only as the mechanical wave (caused by the mixed tones) travels along the cochlear partition upwards and the higher frequency component decays more rapidly than the lower one as they travel (21). The application of Fourier analysis to a complex sound wave and interpretation of the total physiological effect as a sum of the effects of those constituent pure tones is dangerous and in most cases erroneous. Wave analysis is of course essential for any theoretical and practical treatments of dynamical problems in the cochlea; but, for consideration of the process of initiation of nerve impulses (which are all-or-none in nature), particularly in the basal turn where any mixture of tones can act without being separated into its components, mathematical analysis of the complex wave into its physical components is undoubtedly worthless.

Through the experimental results described in this paper the physiological bases for pitch perception in man has also become clearer. Up to 2000 cps at least, nerve impulses in individual auditory nerve fibers tend to appear at some integral multiple of the period of the applied pure tone. In the entire range of the audible sounds, the pattern of distribution of excitatory process in the cochlea (as examined by observing its tendency to initiate nerve impulses) changes as a function of frequency: the lower the frequency the greater is the emphasis in the apical part of the cochlea. A pure tone excites in general an "area," and not a "spot," in the cochlea. A tone is in a sense a complex stimulus that causes responses in many different nerve fibers from different places in the cochlea.

Summary and Conclusion

1. A technique was developed to record single-fiber responses from the cochlear nerve in the modiolus of the guinea pig on acoustic stimulation.

2. Spontaneous discharges of impulses were recorded from many non-auditory nerve fibers in the eighth nerve.

3. Spontaneous discharges of impulses in individual auditory nerve fibers were never inhibited by acoustic stimulation. This is one of the main differences between the primary and the higher neurons in the auditory system.

4. In response to a strong 8000 cps tone pip, single-fiber spikes started at, or slightly after, the peak of the whole-nerve response. These spikes showed a marked variation in latency. The major portion of the N_2-response of the whole-nerve action potential is generated by the repetitive activity of the primary neurons.

5. The nerve fibers arising in the basal turn of the cochlea respond to tones of any audible frequency. The fibers arising in the upper part of the cochlea respond only to low-frequency tones. The response-area was mapped out for several single auditory fibers.

6. The size of the microphonic response recorded with differential electrodes from different parts in the cochlea represents the tendency of a tone to initiate nerve impulses at that place and frequency.

7. With 290 and 500 cps tones the nerve impulses are initiated early in that phase of the microphonic response during which the hair cells are traversed by electric current flowing from scala vestibuli to scale tympani. This strengthens the view that the microphonic responses are direct electrical stimuli for initiating impulses at the nerve endings.

8. Some of the basal turn fibers have higher threshold than others. It is suggested that the nerve endings on the internal hair cells are less sensitive to sounds than those on the external hair cells.

9. Following application of a short strong tone pip a brief after-discharge of impulses was often observed.

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